CHAPTER III

RESULTS

RESULTS

Section - A

Studies on arginase, ornithine ketoacid aminotransferase (OKAT), ornithine carbamyl transferase (OCT) and putrescine carbamyl transferase (PCT) activity in groundnut 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

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

Period of germination (days)	Enzyme units/g fresh tissue			
	GAUG-1	SB-11	Punjab-1	GAUG-10
Cotyledon				
0	17	17	18	17
2	64	67	64	68
4	131	131	138	134
6	123	124	131	128
Embryo				
0	33	34	34	32
2	57	53	58	58
4	35	34	36	34
6	19	21	20	20

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

Table 3 : Ornithine ketoacid aminotransferase activity in different varieties of groundnut seeds during germination.

Period of germination (days)	Enzyme units/g fresh tissue			
	GAUG-1	SB-11	Punjab-1	GAUG-10
Cotyledon				
0	2	3	3	2
2	12	11	13	12
4	20	18	20	19
6	18	18	20	18
Embryo				
0	5	5	5	5
2	15	14	15	15
4	6	6	6	6
6	6	5	6	6

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

Period of germination (days)	Enzyme units/g fresh tissue			
	GAUG-1	SB-11	Punjab-1	GAUG-10
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

Table 5 : Putrescine carbamyl transferase activity in different varieties of groundnut seeds during germination.

Period of germination (days)	Enzyme units/g fresh tissue			
	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	$\angle$0.2	$\angle$0.2	$\angle$0.2
Embryo				
0	11	11	8	8
2	5	6	6	6
4	3	3	3	4
6	2	2	2	2

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

Stage	Average weight of seed (mg)	Dry weight (%)		Protein (mg/g fresh tissue)	
		Cotyledon	Embryo	Cotyledon	Embryo
A. Development (stage)					
1	5	8		11	
2	27	14		49	
3	75	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	105
9	717	74	68	156	131
B. Storage (months)					
6	462	76	69	158	135
12	424	79	71	169	141
C. Germination (days)					
0		70	68	153	128
1		65	57	146	102
2		58	46	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

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

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

Stage	Enzyme units/g fresh tissue		Enzyme units/mg protein	
	Cotyledon	Embryo	Cotyledon	Embryo
A. Development (stage)				
1	30		2.7	
2	29		0.6	
3	26	-	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
B. 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

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

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

Stage	Enzyme units/g fresh tissue		Enzyme units/mg protein	
	Cotyledon	Embryo	Cotyledon	Embryo
A. Development (stage)				
1	7		0.64	
2	6		0.12	
3	5	-	0.10	-
4	5	-	0.09	-
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	$\angle$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

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

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

Stage	Enzyme units/g fresh tissue		Enzyme units/mg protein	
	Cotyledon	Embryo	Cotyledon	Embryo
A. Development (stage)				
1		41		3.7
2		53		1.1
3	65	-	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)				
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	68	0.8	2.8
8	63	67	0.8	3.4

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

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

Stage	Enzyme units/g fresh tissue		Enzyme units/mg protein	
	Cotyledon	Embryo	Cotyledon	Embryo
A. Development (stage)				
1	15		1.36	
2	15		0.31	
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
B. 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	$\angle$0.2	3		0.11
7	0	3		0.13
8	0	3		0.15

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 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 around 3rd day.

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 embryo 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.

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.

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

Age of plant (days)	Enzyme units/g fresh tissue				
	Cotyledon	Hypocotyl	Root	Shoot	Leaves
14	94	29	13	11	35
21	30	25	6	7	19
28	9	5	4	3	8
60			2	4	5

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

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

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

Age of plant (days)	Enzyme units/g fresh tissue				
	Cotyledon	Hypocotyl	Root	Shoot	Leaves
14	63	48	16	38	75
21	36	31	6	33	49
28	16	33	5	30	42
60			5	4	21

Table 14 : Putrescine carbamyl transferase activity from groundnut plants raised in garden soil.

Age of plant (days)	Enzyme units/g fresh tissue				
	Cotyledon	Hypocotyl	Root	Shoot	Leaves
14	2	3	1	2	4
21	2	0	$\angle$0.2	0.6	0.6
28	1	0	$\angle$0.2	0.5	0.6
60			0	0	0

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 and 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

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

Period of germination (days)	Enzyme units/g fresh tissue at 2,4-D concentration (ppm)				
	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

Table 16 : Effect of IAA on arginase activity.

Period of germination (days)	Enzyme units/g fresh tissue at IAA concentration (ppm)				
	0	25	50	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
Embryo					
0	34	32	32	32	29
2	51	53	49	41	37
4	37	48	72	73	104
6	16	19	18	26	74

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

Period of germination (days)	Enzyme units/g fresh tissue at 2,4-D concentration (ppm)				
	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	20	23

Table 18 : Effect of IAA on ornithine ketoacid amino-transferase activity.

Period of germination (days)	Enzyme units/g fresh tissue at IAA concentration (ppm)				
	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

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

Period of germination (days)	Enzyme units/g fresh tissue at 2,4-D concentration (ppm)				
	0	25	50	100	200
Cotyledon					
0	55	55	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

Table 20 : Effect of IAA on ornithine carbamyl transferase activity.

Period of germination (days)	Enzyme units/g fresh tissue at IAA concentration (ppm)				
	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

Table 21 : Effect of 2,4-D on putrescine carbamyl transferase activity.

Period of germination (days)	Enzyme units/g fresh tissue at 2,4-D concentration (ppm)				
	0	25	50	100	200
Cotyledon					
0	7	6	6	6	6
2	4	3	3	2	2
4	3	4	3	3	3
6	<0.2	<0.2	<0.2	<0.2	<0.2
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

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

Period of germination (days)	Enzyme units/g fresh tissue at IAA concentration (ppm)				
	0	25	50	100	200
Cotyledon					
0	7	6	6	6	5
2	4	3	3	3	3
4	3	3	3	2	2
6	∠0.2	∠0.2	∠0.2	∠0.2	∠0.2
Embryo					
0	7	7	7	7	6
2	4	4	4	4	4
4	2	2	2	2	2
6	2	2	3	2	2

Table 23 : Effect of gibberellic acid on arginase activity.

Period of germination (days)	Enzyme units/g fresh tissue at gibberellic acid concentration (ppm)				
	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	68	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

Table 24 : Effect of kinetin on arginase activity.

Period of germination (days)	Enzyme units/g fresh tissue at kinetin concentration (ppm)				
	0	25	50	100	200
Cotyledon					
0	17	17	17	15	9
2	60	27	20	18	14
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

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

Period of germination (days)	Enzyme units/g fresh tissue at gibberellic acid concentration (ppm)				
	0	25	50	100	200
Cotyledon					
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

Table 26 : Effect of kinetin on ornithine ketoacid amino-transferase activity.

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

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

Period of germination (days)	Enzyme units/g fresh tissue at gibberellic acid concentration (ppm)				
	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
Embryo					
0	68	64	61	58	56
2	75	65	63	61	59
4	76	64	61	58	55
6	74	72	72	69	54

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

Period of germination (days)	Enzyme units/g fresh tissue at kinetin concentration (ppm)				
	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	70	70	68	67
4	76	71	68	66	61
6	74	67	63	63	61

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

Period of germination (days)	Enzyme units/g ffesh tissue at gibberellic acid concentration (ppm)				
	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	∠0.2	∠0.2	∠0.2	∠0.2	∠0.2
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 30 : Effect of kinetin on putrescine carbamyl transferase activity.

Period of germination (days)	Enzyme units/g fresh tissue at kinetin concentration (ppm)				
	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	∠0.2	∠0.2	∠0.2	∠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

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

Table 31 : Effect of ethrel on arginase activity.

Period of germination (days)	Enzyme units/g fresh tissue at ethrel concentration (ppm)					
	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

Table 32 : Effect of chloroethanol on arginase activity.

Period of germination (days)	Enzyme units/g fresh tissue at chloroethanol concentration (ppm)					
	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	28
2	53	53	41	37	37	34
4	37	94	95	101	107	88
6	19	42	70	88	91	116

Table 33 : Effect of ethrel on ornithine ketoacid amino-transferase activity.

Period of germination (days)	Enzyme units/g fresh tissue at ethrel concentration (ppm)					
	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 34 : Effect of chloroethanol on ornithine ketoacid aminotransferase activity.

Period of germination (days)	Enzyme units/g fresh tissue at chloroethanol concentration (ppm)					
	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
Embryo						
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

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

Period of germination (days)	Enzyme units/g fresh tissue at ethrel concentration (ppm)					
	0	200	400	600	800	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	69	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

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

Period of germination (days)	Enzyme units/g fresh tissue at chloroethanol concentration (ppm)					
	0	200	400	600	800	1000
Cotyledon						
0	58	58	58	58	58	57
2	64	62	61	61	61	61
4	66	64	63	59	54	50
6	64	64	63	60	58	54
Embryo						
0	69	69	69	69	69	69
2	76	61	58	58	55	55
4	74	72	68	66	65	63
6	72	70	70	66	66	63

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

Period of germination (days)	Enzyme units/g fresh tissue at ethrel concentration (ppm)					
	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	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Embryo						
0	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

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

Period of germination (days)	Enzyme units/g fresh tissue at chloroethanol concentration (ppm)					
	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	∠0.2	∠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

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

Table 39 : Effect of polyamines on arginase activity.

Period of germination (days)	Enzyme units/g fresh tissue with polyamine (1000 ppm)			
	None	Putrescine	Spermidine	Spermine
Cotyledon				
0	17	17	17	17
2	58	59	59	57
4	137	137	136	133
6	145	141	145	147
Embryo				
0	33	33	33	33
2	61	80	86	78
4	35	64	49	61
6	17	33	40	30

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

Period of germination (days)	Enzyme units/g fresh tissue with polyamine (1000 ppm)			
	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

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

Period of germination (days)	Enzyme units/g fresh tissue with polyamine (1000 ppm)			
	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
Embryo				
0	70	70	65	65
2	75	73	73	71
4	71	70	71	71
6	73	71	71	71

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

Period of germination (days)	Enzyme units/g fresh tissue with polyamine (1000 ppm)			
	None	Putrescine	Spermidine	Spermine
Cotyledon				
0	6	6	5	5
2	4	4	4	4
4	2	2	2	2
6	<0.2	<0.2	<0.2	<0.2
Embryo				
0	7	7	7	7
2	4	4	4	4
4	3	3	2	2
6	2	2	2	2

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

Treatment*	Enzyme units/g fresh tissue at hour of incubation					
	0	1	2	3	4	6
Cotyledon						
-	137	32	16	16	13	5
2,4-D	93	24	12	12	11	3
Chloro-ethanol	26	7	3	3	3	1
Embryo						
-	35	13	7	7	7	3
2,4-D	90	30	18	16	14	7
Chloro-ethaml	84	29	17	14	14	5

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

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

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

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

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.

Treatment	Concentration (ppm)	Enzyme units/g fresh tissue			
		Cotyledon		Embryo	
		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	2000 + 10	5	6	36	22
IAA	100	91	81	86	42
IAA + cycloheximide	100 + 10	58	41	42	24
IAA	200	80	78	96	61
IAA + cycloheximide	200 + 10	36	42	34	22

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

Treatment	Concentration (ppm)	Enzyme units/g fresh tissue			
		Cotyledon		Embryo	
		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
Chloroethanol	2000	2	4	12	18
Chloroethanol + cycloheximide	2000 + 10	1	1	9	6
IAA	100	14	17	17	18
IAA + cycloheximide	100 + 10	8	10	8	6
IAA	200	2	15	9	25
IAA + cycloheximide	200 + 10	1	6	4	6

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

Treatment	Concentration (ppm)	Enzyme units/g fresh tissue			
		Cotyledon		Embryo	
		4th day	6th day	4th day	6th day
-	-	137	145	35	17
Cyclohexiimide	10	138	147	35	18
Putrescine	1000	137	141	64	33
Putrescine + cyclohexiimide	1000 + 10	126	144	35	19
Spermidine	1000	136	145	49	40
Spermidine + cyclohexiimide	1000 + 10	128	140	34	19
Spermine	1000	133	147	61	30
Spermine + cyclohexiimide	1000 + 10	124	146	32	16

Table 48 : Effect of cycloheximide on ornithine ketoacid aminotransferase activity in presence of polyamines on 4th and 6th day of germination.

Treatment	Concentration (ppm)	Enzyme units/g fresh tissue			
		Cotyledon		Embryo	
		4th 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.

Treatment	Concentration (ppm)	Enzyme units/g fresh tissue			
		Cotyledon		Embryo	
		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	91	81	86	42
IAA + 5-Fluorouracil	100 + 10	86	72	78	41

Table 50 : Effect of 5-Fluorouracil on ornithine ketoacid aminotransferase activity in presence of chloroethanol and IAA on 4th and 6th day of germination.

Treatment	Concentration (ppm)	Enzyme units/g fresh tissue			
		Cotyledon		Embryo	
		4th day	6th day	4th day	6th day
-	-	25	23	6	5
5-Fluorouracil	10	25	23	06	05
Chloroethanol	1000	4	21	19	25
Chloroethanol + 5-Fluorouracil	1000 + 10	4	16	17	22
IAA	100	14	17	17	18
IAA + 5-Fluorouracil	100 + 10	12	17	15	16

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 influenced by the cotyledons, the embryo was cultivated on a synthetic medium as described in Materials and Methods. 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 those used for whole seeds.

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

Treatment	Concentration (ppm)	Enzyme units/g fresh tissue period of cultivation (days)			
		0	2	4	6
-	-	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

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

Treatment	Concentration (ppm)	Enzyme units/g fresh tissue period of cultivation (days)			
		0	2	4	6
-	-	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

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

Period of cultivation (days)	Enzyme units/g fresh tissue with polyamine (250 ppm)			
	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 54 : Effect of polyamines on ornithine ketoacid aminotransferase activity of the embryo maintained in vitro.

Period of cultivation (days)	Enzyme units/g fresh tissue with polyamine (250 ppm)			
	None	Putrescine	Spermidine	Spermine
0	5	5	5	6
2	15	17	17	18
4	7	11	13	15
6	6	11	12	11

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

Treatment	Concentration (ppm)	Enzyme units/g fresh tissue			
		Arginase		OKAT	
		4th day	6th day	4th day	6th day
-	-	36	16	6	5
Cyclohexiimide	2.5	37	17	6	5
2,4-D	25	95	89	13	13
2,4-D + cyclohexiimide	25 + 2.5	38	19	7	5
Chloroethanol	400	107	103	14	15
Chloroethanol + cyclohexiimide	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.

Treatment	Concentration (ppm)	Enzyme units/g fresh tissue			
		Arginase		OKAT	
		4th day	6th day	4th day	6th day
-	-	38	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	75	33	15	11
Spermine + cycloheximide	250 + 2.5	36	18	6	6

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

Table 57 : Purification of groundnut cotyledon arginase.

Purification step	Total volume (ml)	Total activity (units)	Total protein (mg)	Specific activity (units/mg protein)	Purification (fold)	Recovery (%)
Homogenate	210	2340	2360	1.0	1	100
Supernatant	203	2270	1980	1.2	1	97
Heat treated supernatant	200	2240	1500	1.5	2	96
DEAE eluate	200	2080	360	5.8	9	89
Alumina Cγ gel eluate	200*	2080	240	8.7	9	89
Ist ammonium sulphate fraction	120	1610	36	44.7	45	69
DEAE-Sephadex eluate	120	1450	21	69.0	69	62
Calcium phosphate gel eluate	120*	1340	12	111.6	111	57
IInd ammonium sulphate fraction	73	900	6	150.0	150	38

* corrected volume after dialysis.

Table 58 : Purification of groundnut embryo arginase.

Purification step	Total volume (ml)	Total activity (units)	Total protein (mg)	Specific activity (units/mg protein)	Purification (fold)	Recovery (%)
Homogenate	100	620	750	0.8	1	100
Supernatant	98	620	640	1.0	1	99
Ist ammonium sulphate fraction	65	370	200	1.9	2	60
DEAE eluate	66	310	50	6.2	8	49
Alumina C_γ gel eluate*	66*	300	30	10.0	13	49
IInd ammonium sulphate fraction	39	250	14	17.8	22	49
Calcium phosphate gel eluate	39*	220	7	31.4	40	36

* 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°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.

Table 59 : Effect of storage on arginase activity.

Period of storage (days)	µmoles of ornithine formed	
	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

* Enzyme were stored at 5-10^{o}C in refrigerator.

Table 60 : Effect of pH on arginase activity.

pH*	µmoles of ornithine formed	
	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. Tris-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

* 50 µmoles of buffer was used.

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

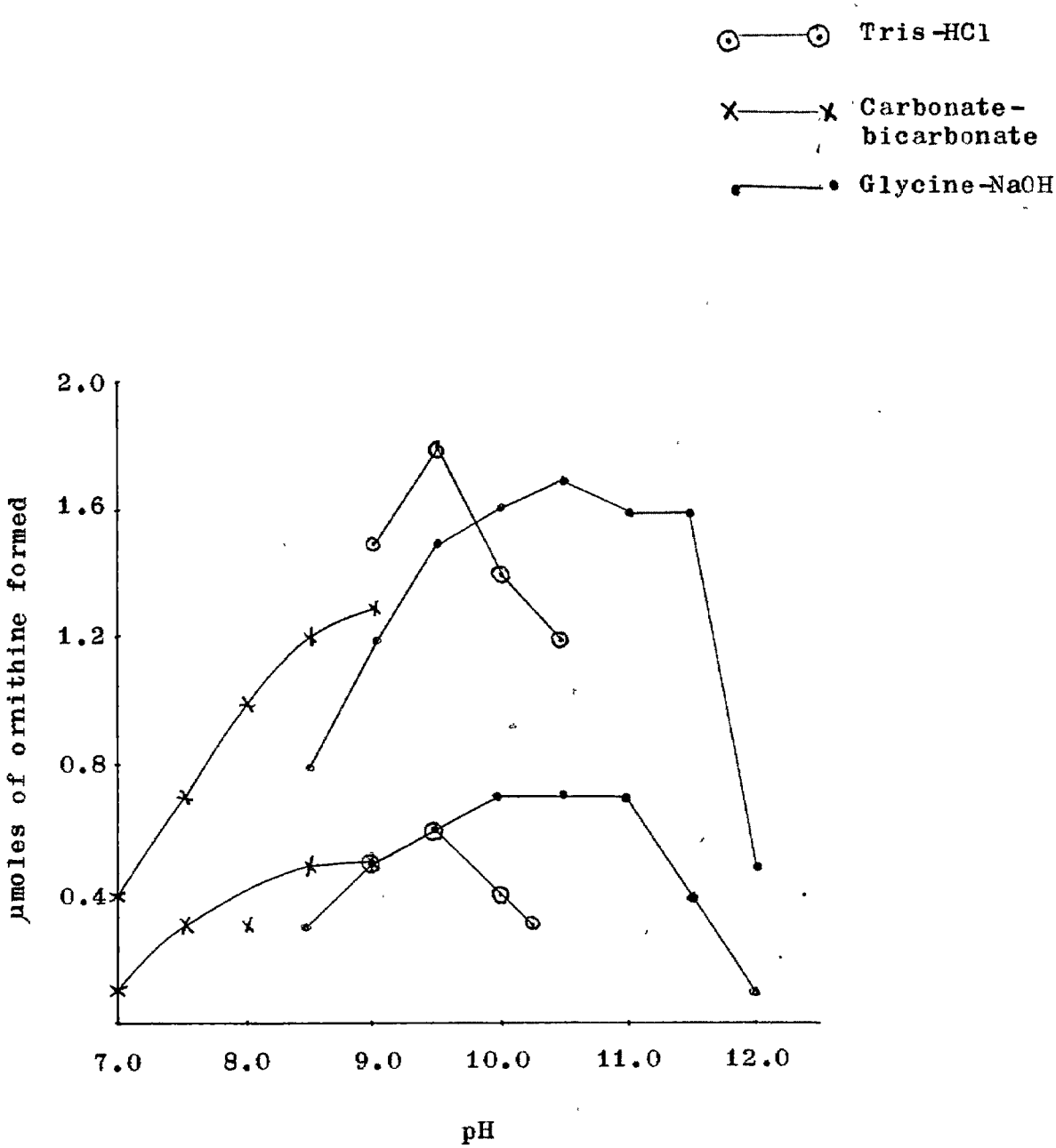

Fig. 2 : Effect of pH on arginase activity.

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

Enzyme concentration (µg protein)	µmoles 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

The purified enzyme was diluted 1:1 with 5 mM Na-K-PO_4 buffer, pH 7 containing 10 mM mercaptoethanol and 1 x 10^{-4} mM $MnCl_2$ before use.

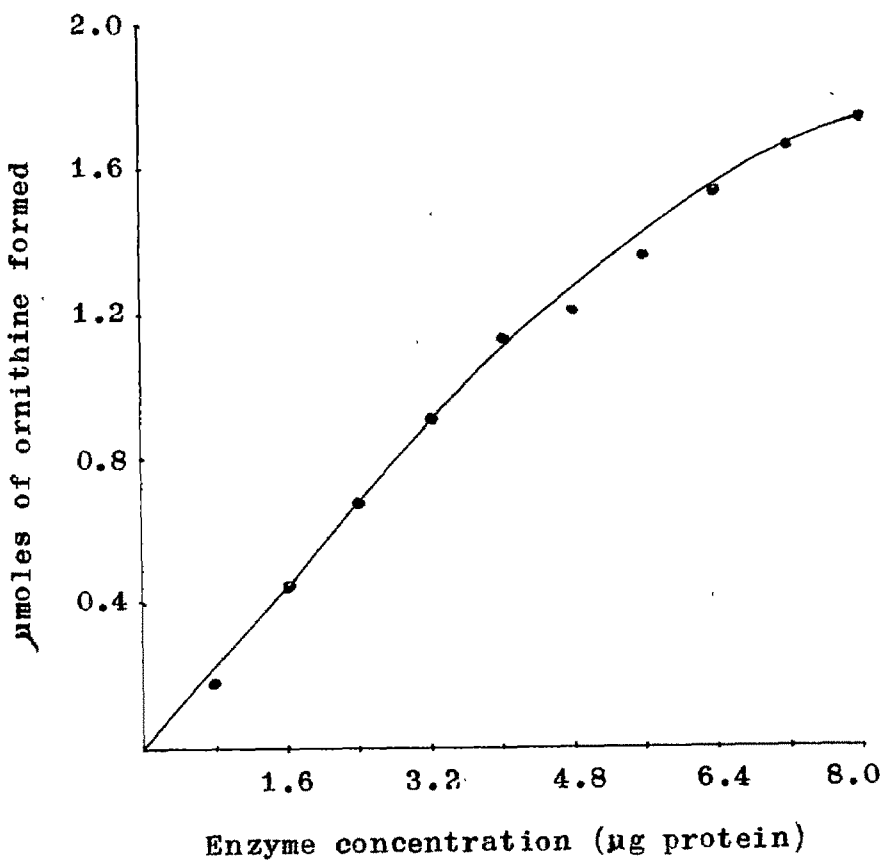

Fig. 3 : Effect of enzyme concentration on cotyledon arginase activity.

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

Enzyme concentration (µg protein)	µmoles 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

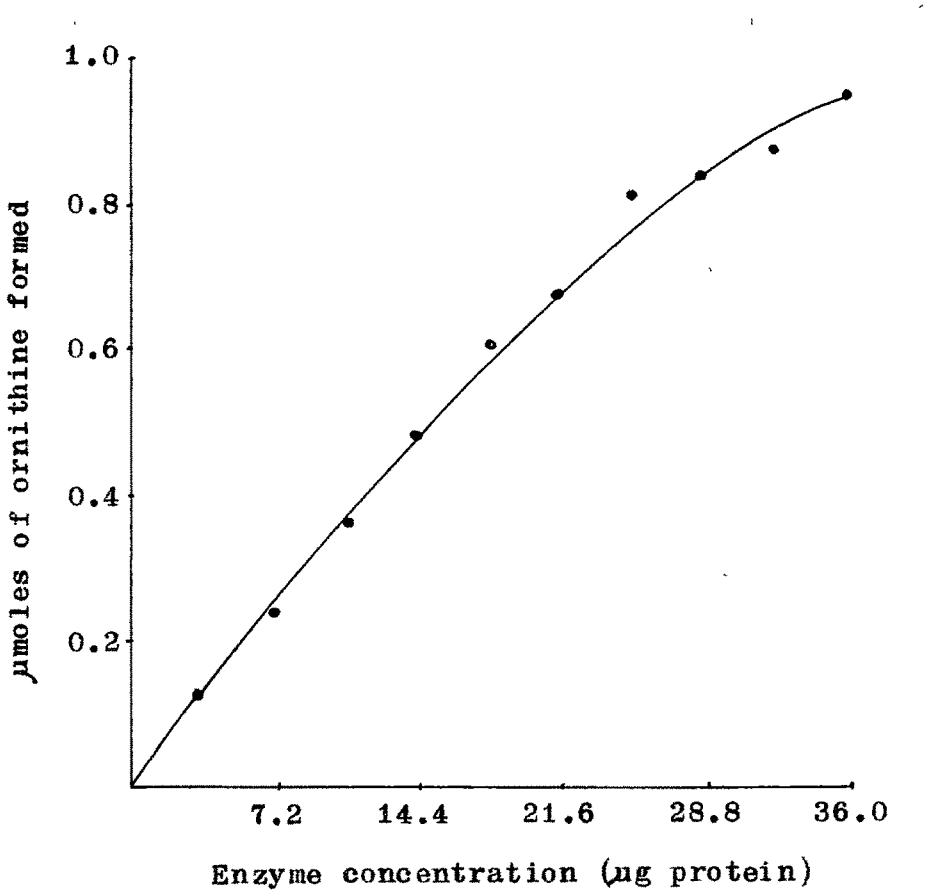

Fig. 4 : Effect of enzyme concentration on embryo arginase activity.

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°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°C for cotyledon enzyme and 11.554 Kcal/mole and 2.152 Kcal/mole with a transition temperature of 23.7°C for embryo enzyme at pH 9.5.

Heat stability

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.

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

Period of incubation (min)	µmoles of ornithine formed	
	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

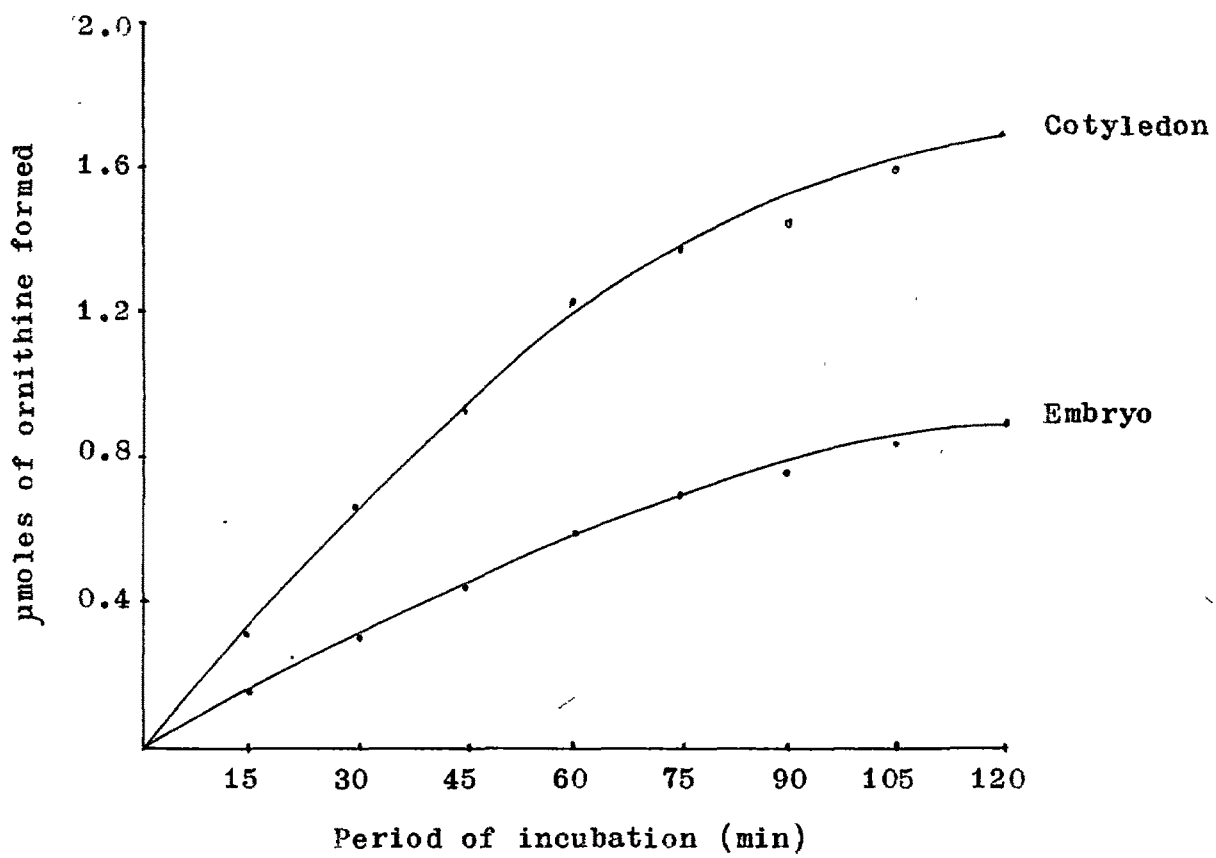

Fig. 5 : Effect of period of incubation on arginase activity.

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

Arginine (µmoles)	µmoles of ornithine formed	
	Experiment - I	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 65 : Effect of substrate concentration on embryo arginase activity.

Arginine (µmoles)	µmoles of ornithine formed	
	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 66 : Km value for cotyledon and embryo arginase from the data given in Tables 64 and 65.

	Km (mM)	Mean Km (mM)
Cotyledon :		
Experiment - I	5.0	
		5.8
Experiment - II	6.6	
Embryo :		
Experiment - I	5.6	
		5.6
Experiment - II	5.6	

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

Incubation temperature (^{o}C)	µmoles of ornithine formed	
	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

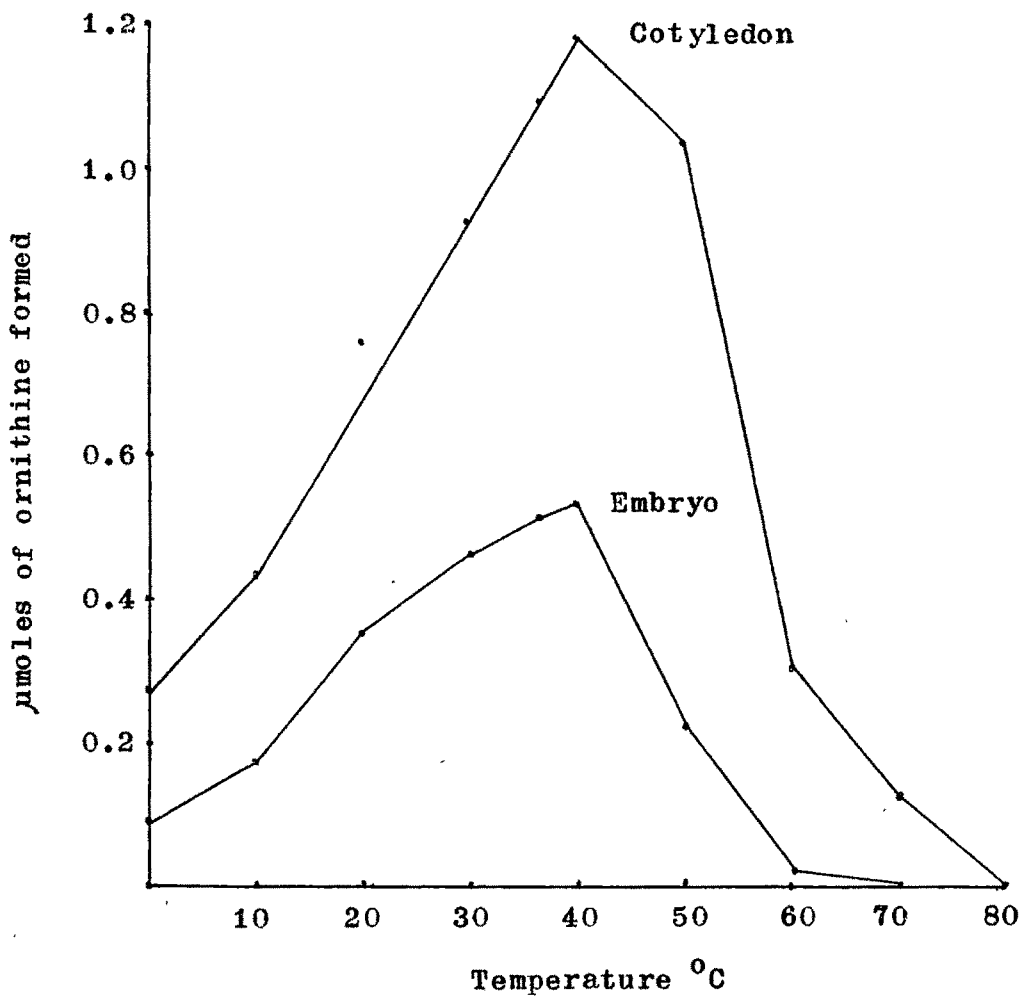

Fig. 6 : Effect of temperature of incubation on arginase activity.

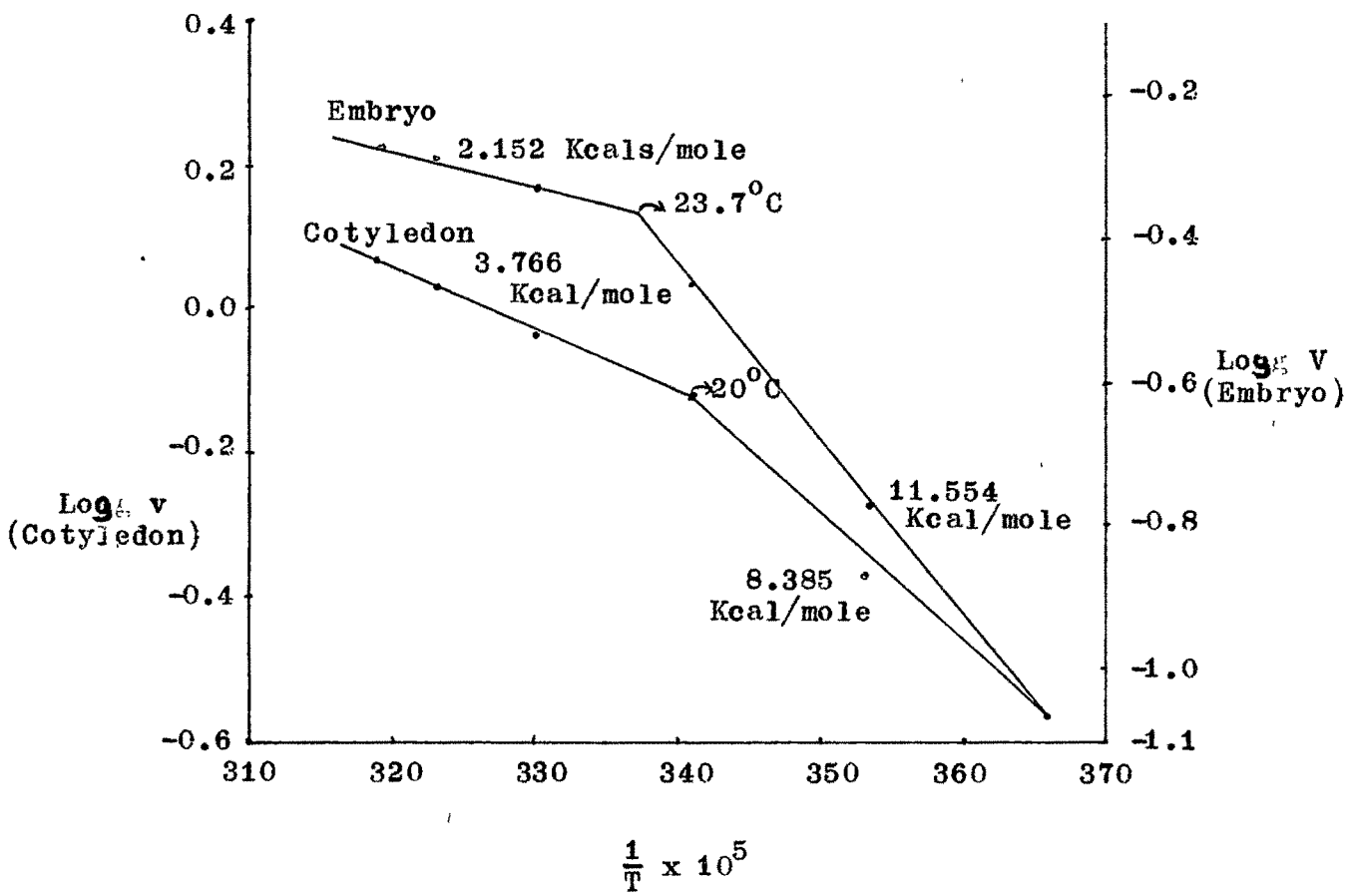

Fig. 7 : Arrhenius plot for arginase activity.

Table 68 : Effect of heat inactivation on arginase activity.

Temperature of inactivation* (°C)	µmoles of ornithine formed	
	Cotyledon	Embryo
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)

* 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.

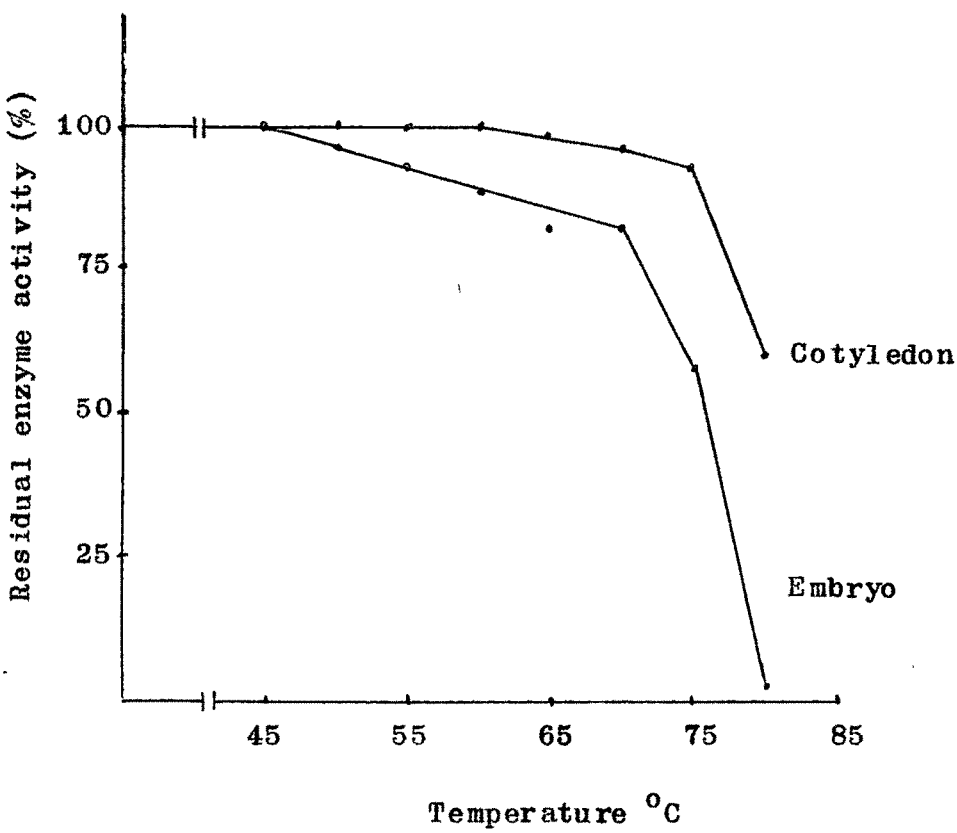

Fig. 8 : Effect of heat inactivation on arginase activity.

Section - C

Purification and properties of ornithine and putrescine carbamyl transferase from groundnut cotyledon

The results reported in Section A showed the presence of putrescine carbamylating activity in both cotyledons and embryos during 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.

Table 69 : Purification of ornithine and putrescine carbamyl transferase activity.

Purification step	Total volume ml	Total units		Total protein (mg)	units/mg protein		Purification (fold)		Yield (%)		OCT/PCT
		OCT	PCT		OCT	PCT	OCT	PCT	OCT	PCT	
Homogenate	300	3010	230	8100	0.37	0.03	1	1	100	100	13.5
Supernatant	268	2900	220	4620	0.62	0.05	2	2	96	95	13.2
Ammonium sulphate fraction	157	1930	120	2260	0.85	0.05	2	2	64	51	16.1
pH supernatant	162	1940	110	160	12.12	0.68	33	22	64	48	17.6
Alumina Cr gel eluate	184	1780	80	80	22.13	1.00	61	33	59	34	22.1
DEAE eluate	95	1040	40	10	103.00	3.72	278	133	34	18	25.8

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.

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 ornithine, putrescine and carbamyl phosphate as variable substrates at a fixed concentration of

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

pH*	µmoles of citrulline/N-carbamyl putrescine formed	
	OCT	PCT
I. 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
II. 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
III. 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

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

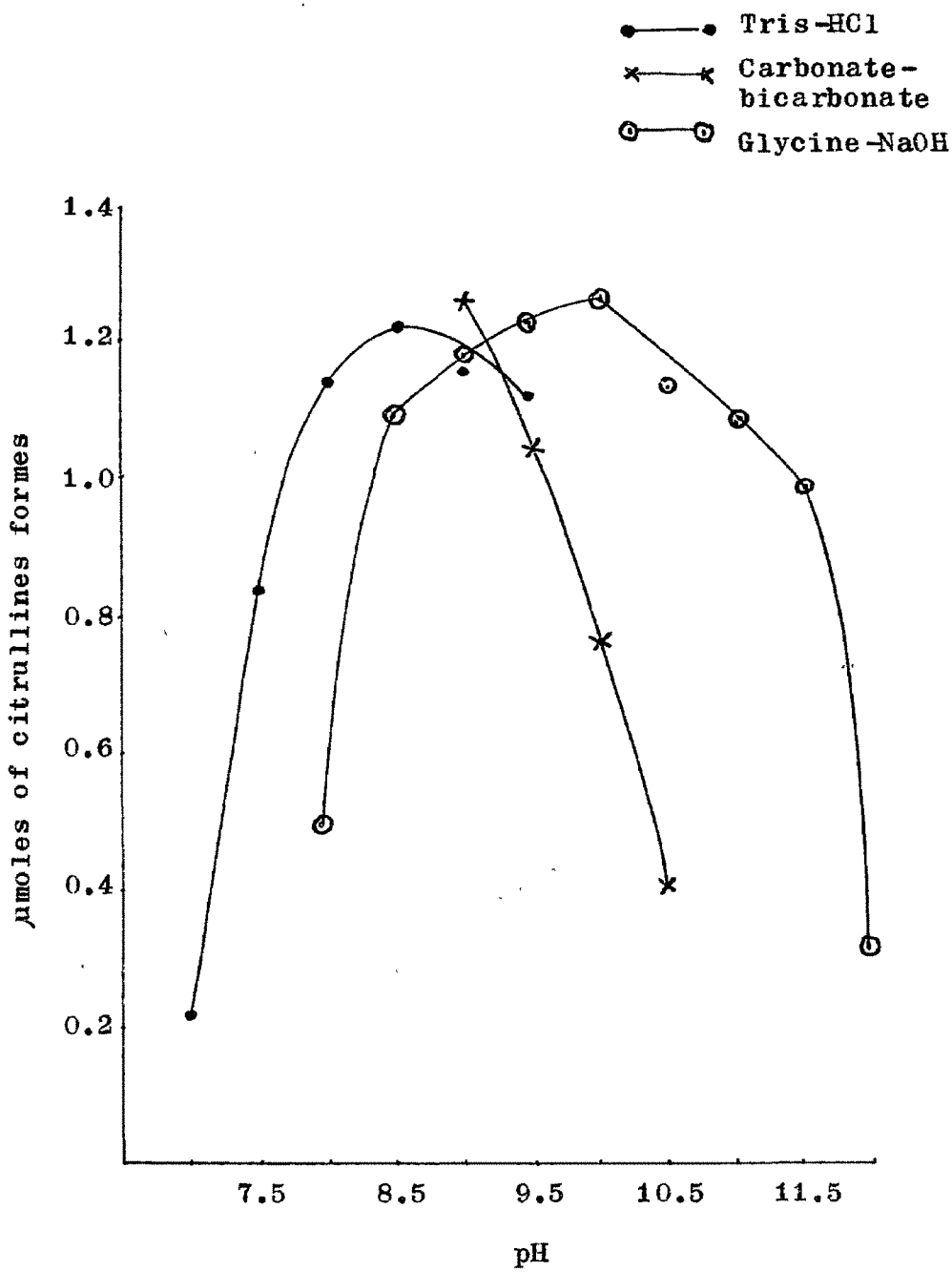

Fig. 9 : Effect of pH on OCT activity.

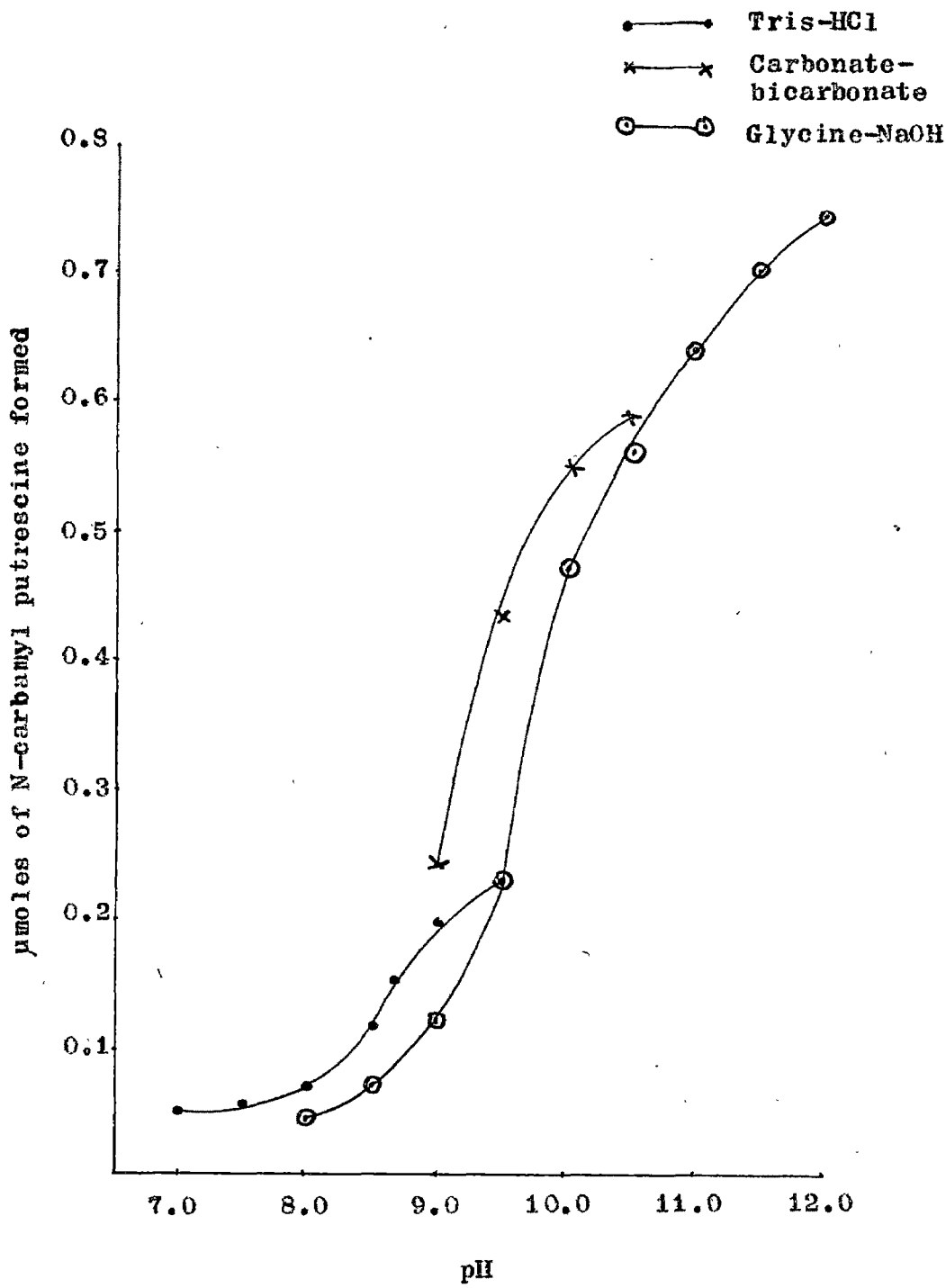

Fig. 10 : Effect of pH on PCT activity.

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

Enzyme concentration (µg protein)	µmoles of citrulline formed
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

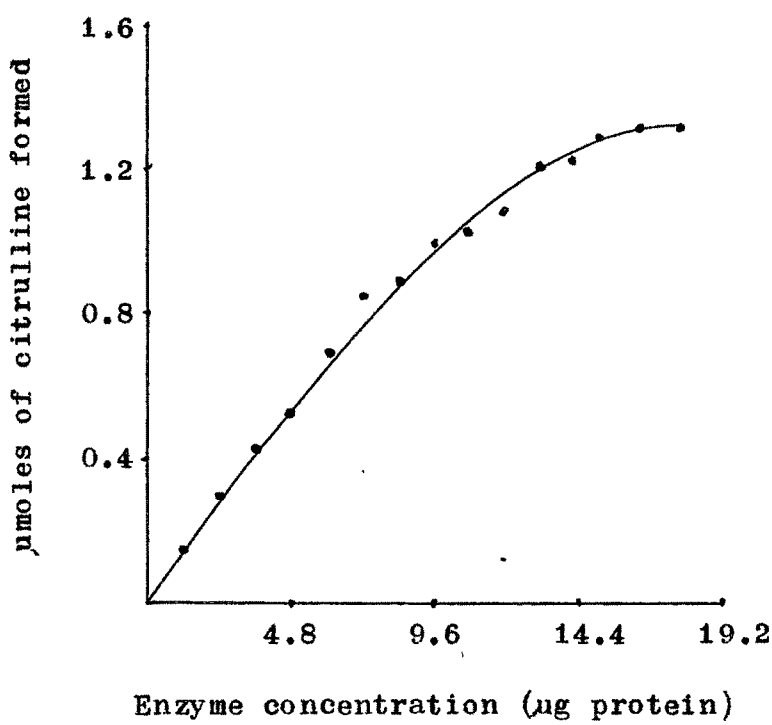

Fig. 11 : Effect of enzyme concentration on OCT activity.

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

Enzyme concentration (µg protein)	µmoles 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

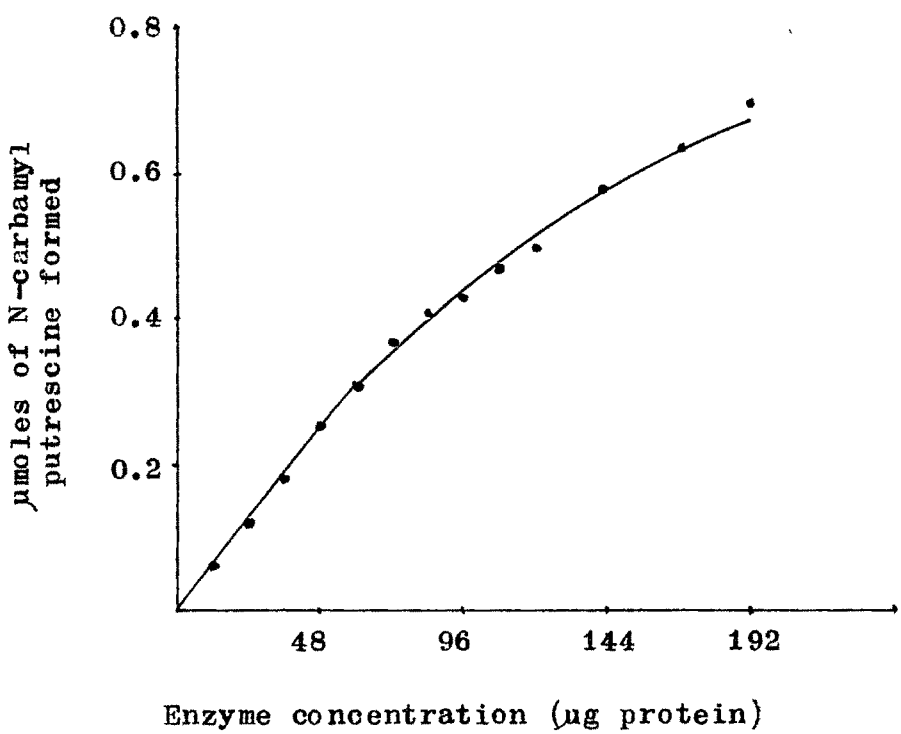

Fig. 12 : Effect of enzyme concentration on PCT activity.

Table 73 : Effect of period of incubation on ornithine and putrescine carbamyl transferase activity.

Incubation time (min)	µmoles of citrulline/ N-carbamyl putrescine formed	
	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

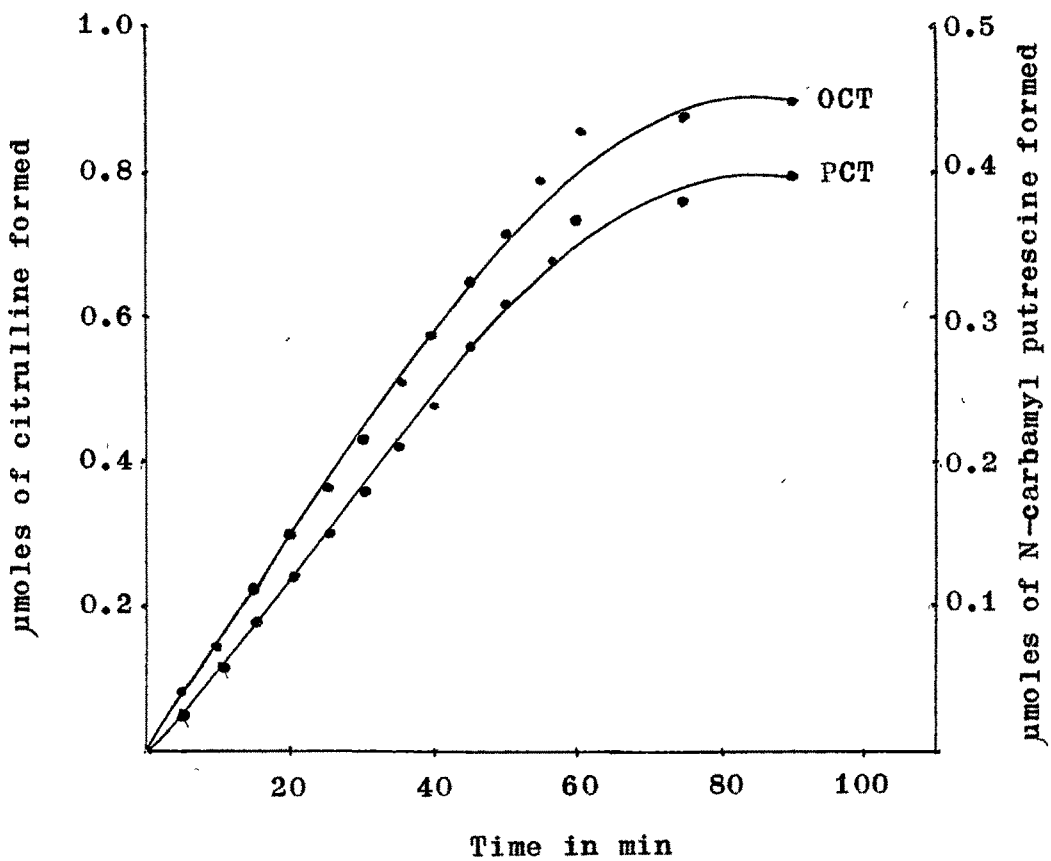

Fig. 13 : Effect of period of incubation on OCT and PCT activity.

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

Varied substrate concentration (umoles)	µmoles of citrulline formed with variable substrate			
	Ornithine		Carbamyl phosphate	
	Experiment I	Experiment II	Experiment I	Experiment 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
3.4	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

* Concentration of non-varied substrate was 5 µmoles.

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

Putrescine concentration (umoles)	µmoles of N-carbamyl putrescine formed	
	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

Concentration of carbamyl phosphate was 5 µmoles.

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

Carbamyl phosphate concentration* (umoles)	µmoles of N-carbamyl putrescine formed	
	Experiment - I	Experiment - II
0.05	0.02	0.02
0.10	0.03	0.03
0.15	0.05	0.04
0.20	0.06	0.06
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

* Concentration of putrescine was 5 µmoles.

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

Substrate	Km (mM)	Mean Km (mM)
For OCT		
Ornithine :		
Experiment - I	1.22	
		1.16
Experiment -- II	1.10	
Carbamyl phosphate :		
Experiment - I	0.18	
		0.21
Experiment - I	0.23	
For PCT		
Putrescine :		
Experiment - I	4.02	
		4.03
Experiment - II	4.05	
Carbamyl phosphate :		
Experiment - I	0.19	
		0.19
Experiment - II	0.19	

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°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°C for 10 min, whereas OCT retained 100% activity under similar conditions. Both the enzymes were completely inactivated at 70°C.

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

Temperature of incubation (^{o}C)	µmoles of citrulline/N-carbamyl putrescine formed	
	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

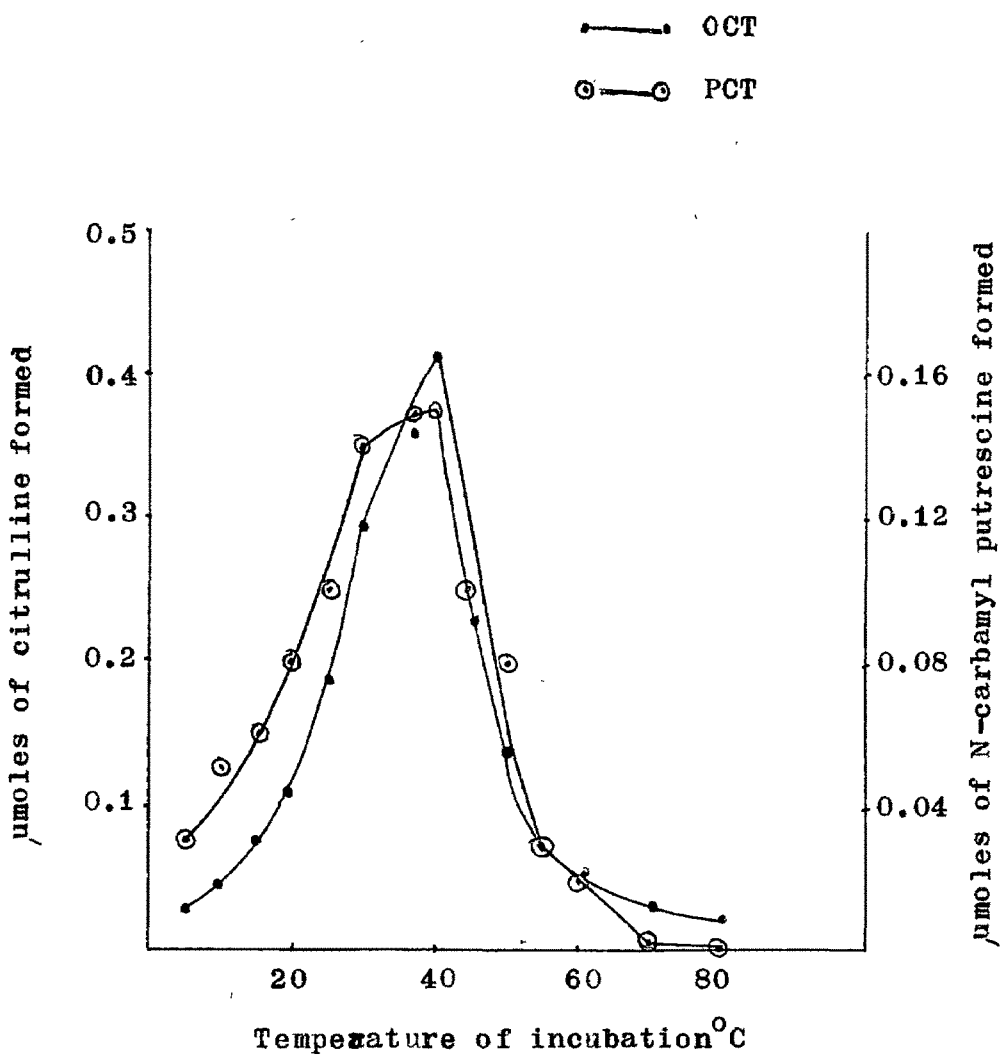

Fig. 14 : Effect of temperature of incubation on OCT and PCT activity.

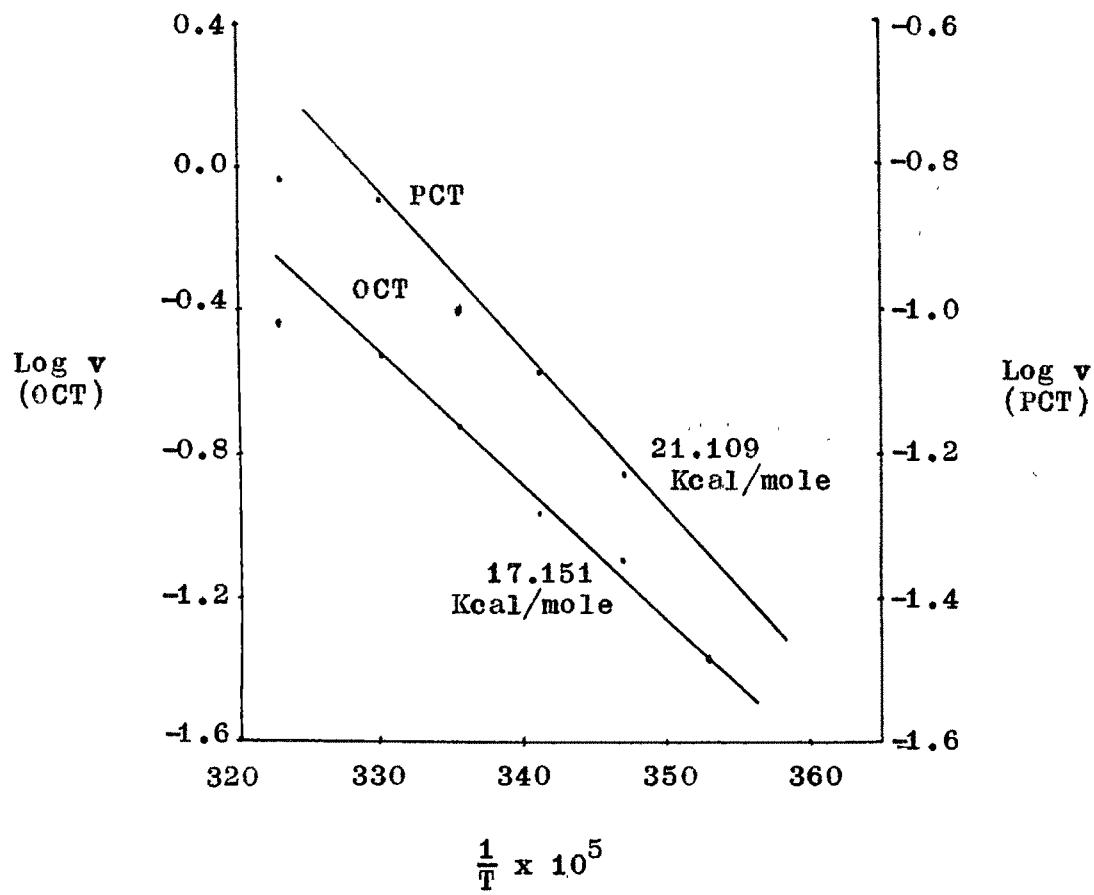

Fig. 15 : Arrhenius plot for OCT and PCT activity.

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

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

* 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^{o}C.

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

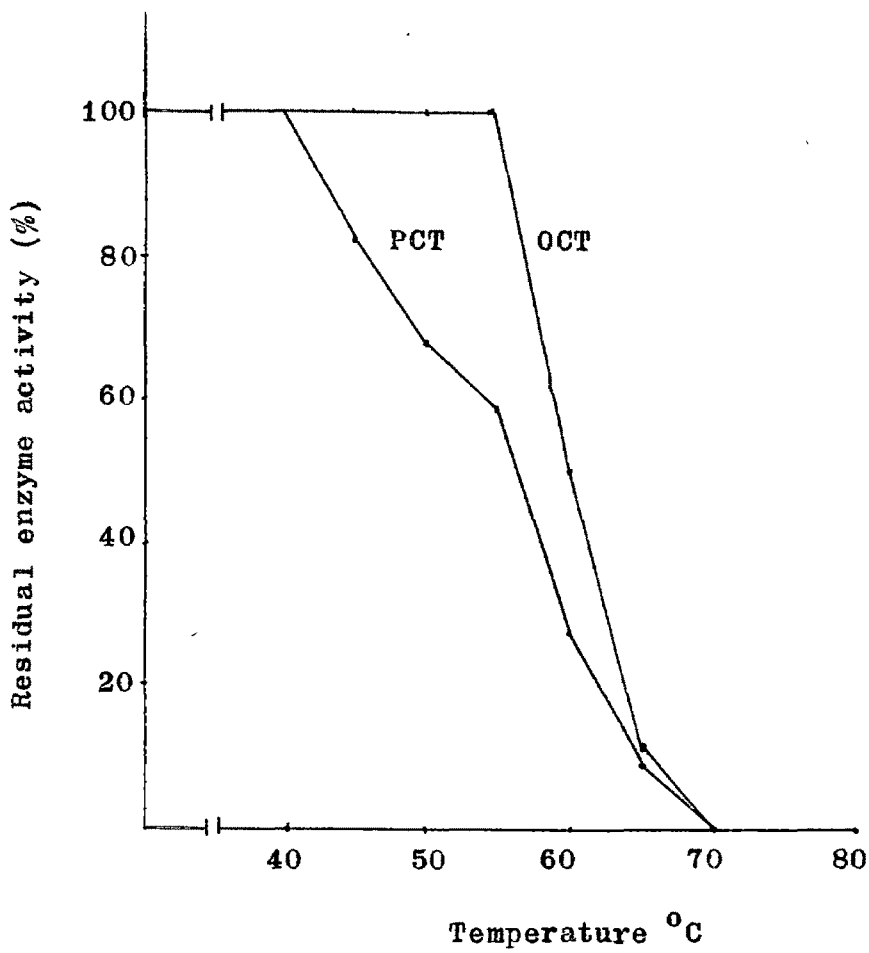

Fig. 16 : Effect of heat inactivation on OCT and PCT activity.