transfer from parent to daughter cells. Some loss of virus during mitosis was not ruled out. TMV-containing cells may not have divided, but this does not seem likely. The role of mitosis in TMV metabolism in other tissues is only suggested by these data. If iodine is transferred from virus to spleen protein, the true disappearance rate of the virus in spleen is even greater than measured.

The possibility of deiodination of intact virus has not been dismissed. *In vitro* experiments of Tong *et al.*(11) indicate that liver can deiodinate diiodotyrosine, but spleen has little or no such ability. The same may be true for deiodination of the virus.

Summary. Rate of disappearance of injected I^{131}-labeled TMV was more rapid from spleen, an important site of antibody production, than from liver, which produces little or no antibody. It was concluded, therefore, that persistence of the virus is not in direct relation to the tissue's antibody-producing capacity. The need for studies at cellular level was indicated. In regenerating liver, total radioactivity was not significantly influenced by increased mitotic rate. This was interpreted as indicating that virus localized in liver may be retained during cell division.

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Synthesis of Glucose-Cyclo-Acetoacetate and Biosynthesis of Ascorbic Acid in Germinating Mung Beans (*Phaseolus radiatus*).* (24414)

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After Nath *et al.*(1) reported that acetoacetate accelerates biosynthesis of ascorbic acid in rats, it was shown that glucose condensed with acetoacetate, can give rise to formation of ascorbic acid in germinating legumes (*Phaseolus radiatus*)(2) and in rats (3). An alternative pathway of conversion of glucose to ascorbic acid has been suggested by Horowitz and King(4) and Isherwood *et al.*(5) in chloretinised rats and germinating seeds respectively. It has been suggested that formation of d-glucuronolactone is an intermediary stage in such transformation. Chel-

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delin and King(6) have, however, put forward arguments which can detract from the overall importance of our scheme. Thangamani and Sarma(7) recently have shown that the yield of radioactive l-ascorbic acid in germinating mung beans from labelled glucose-cyclo-acetoacetate is more than 5 times higher than that from labelled glucose. It has, therefore, been desirable to see if there is any indication of enzymatic synthesis of glucose-cyclo-acetoacetate during the process of germination. Nath and Sahu(8) have shown that glucose occurs in combination with acetoacetate in urine of normal rats thus indicating its formation in the living system. We reported that the condensation product of glu-

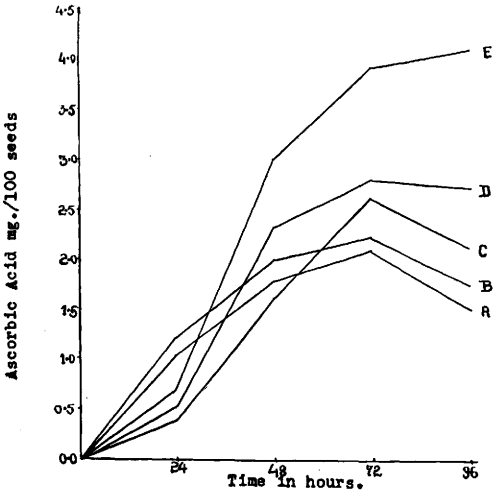

FIG. 1. Effect of glucose, acetoacetate and glucose-cyclo-acetoacetate on the biosynthesis of ascorbic acid in *Phaseolus radiatus*. A—Distilled water. B—Glucose (100 mg/10 cc medium). C—Acetoacetate (70 mg/10 cc medium). D—Acetoacetate (70 mg) + glucose (100 mg) mixture/10 cc medium. E—Glucose cyclo-acetoacetate (100 mg/10 cc).

cose and acetoacetate when hydrolysed with 2N HCl yields a reducing sugar(9) which has been identified as dienol glucose(10).

Methods. Mung beans were first surface sterilized by washing with 0.1% $HgCl_2$ for 3 minutes, washed under aseptic condition with sterile distilled water, then soaked in it for 3 hours prior to addition of different media. Equal number of seeds were transferred to sterile Petri dishes containing different media at pH 7.2. To prevent ascorbic acid forming from auto-oxidization during germination, potassium metabisulphite (300 PPM) was added to all media as recommended by Kendrick and Downer(11). Germination proceeded for 72 hours in sterile chamber protected against direct sunlight. One hundred seedlings were removed of their seed coats, then macerated in metaphosphoric-acetic acid mixture and free ascorbic acid was estimated from the extract according to the method of Bessay and King(12). For glucose, the same procedure was adopted, except that the seeds were thoroughly washed, then macerated only in distilled water and estimation was done according to the method of Nelson(13).

Results. Fig. 1 shows the effect of acetoacetate (sodium salt) with glucose and glucose-cyclo-acetoacetate on biosynthesis of ascorbic acid. Glucose and acetoacetate, present simultaneously in germinating media, accelerate biosynthesis to a greater extent than either of the 2 added individually. Higher value of ascorbic acid is noticed with condensation product of glucose and acetoacetate.

Data in Table I show that values of free glucose are much lower than those of combined glucose. Values of combined glucose are highest when both glucose and acetoacetate are added to the germinating medium. Amounts of free glucose at all stages are much higher when glucose alone is used; but these values are reduced considerably while acetoacetate is present in the germinating medium, thus establishing the possibility of combination of glucose with acetoacetate during germination. Some glucose is known to be released from carbohydrates present in seeds during germination(14). This accounts for the high value of combined glucose in presence of acetoacetate alone.

Results in Tables II and III show that condensation of glucose with acetoacetate is much higher in presence of added thiamine and pantothenic acid and much less when their antivitamins are present in the media.

Summary. Glucose - cyclo - acetoacetate

TABLE I. Effect of Glucose and Acetoacetate on Free and Combined Glucose Present in *Phaseolus radiatus* during Progress of Germination.

Substances in germinating media	Amt in mg/10 cc	Time in hr: 24 hr		48 hr		72 hr	
		Glucose, mg/100 seeds					
		Free	Combined	Free	Combined	Free	Combined
None		5	70	57	53	130	43
Glucose	100	11	79	60	73	146	59
Acetoacetate	70	2.5	117.5	3.5	180.5	5	108
" and glucose	70 + 100	3.5	121	4	196	6.5	183.5

TABLE II. Effect of Antivitamins on Combined Glucose in Germinating Mung Beans in Presence of Glucose and Acetoacetate.

Substances and amt in mg/10 cc medium	Time in hr: 24 hr		48 hr		72 hr	
	Glucose, mg/100 seeds					
	Free	Combined	Free	Combined	Free	Combined
Acetoacetate (70) + glucose (100)	3.5	126.5	12	178	20	156
Idem + Pantoyltaurine (2)	3.1	97.9	8	132	11	122
Acetoacetate (70) + glucose (100)	4	135	13	164	25	200
Idem + pyrithiamine (2)	3.5	130.5	12	157	20	195

TABLE III. Effect of Added Vitamins on Combined Glucose in Germinating Mung Beans in Presence of Acetoacetate and Glucose.

Substances and amt in mg/10 cc medium	Time in hr: 24 hr		48 hr		72 hr	
	Glucose, mg/100 seeds					
	Free	Combined	Free	Combined	Free	Combined
None	7	67	47	59	125	43
Acetoacetate (70) + glucose (100)	5	130	14	184	22.5	187
Idem + thiamine (1)	4.5	161	5	223	7.5	201
" + calcium pantothenate (1)	6.2	159	7	214	8.5	196

(G.C.A.), a precursor of ascorbic acid in germinating legumes and which on hydrolysis gives rise to a reducing dienol glucose, is formed during germination from glucose and acetoacetate. Thiamine and pantothenic acid accelerated biosynthesis of GCA and their antivitamins depressed such synthesis. GCA has thus been shown to be an intermediary product towards biosynthesis of ascorbic acid during germination of legumes.

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