to 1:80 produced positive "surface fixation" tests of different intensity. These discrepancies may be due to inhibitory factors in the agglutination test which have no influence on the paper test.

It may be noticed that stronger "surface fixation" tests are to be found in sera of titres above 1:100. On the other hand, 2 out of 81 cases with significant tube agglutination tests failed to produce "surface fixation" reaction. The only possible explanation of the latter discrepancy may be related to the rather low sensitivity of the paper to sera with agglutinin titres close to 1:100.

Summary. Attempts have been made to standardize the "surface fixation" test for the diagnosis of brucellosis and at the same time make it a practical rapid and dependable procedure. So far, it has been found to possess remarkable specificity in human brucellosis as judged by comparison with blood cultures. This test may be applicable to the detection of bovine reactors.

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Biosynthesis of Vitamin C: A New Precursor. (20258)

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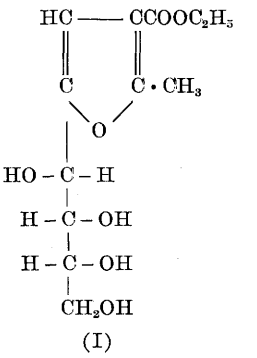
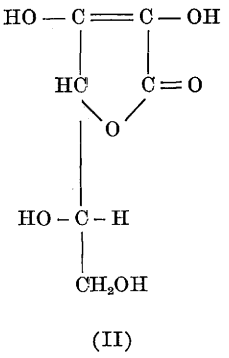
Much work has been done on the biosynthesis of vit. C, but the precise mechanism is still to be revealed. Ray(1), Smythe, and King(2) and several others have indicated that hexose sugars and sugar acids are in some way concerned in the biosynthesis, and according to Mentzer and Urbain(3) lipids also can serve as the precursor of ascorbic acid in the body of the rat.

Acetoacetate, an intermediary metabolite of lipids found by Nath and associates(4,5) to cause an immediate rise in the blood glucose and decrease in glucose tolerance, has recently been reported(6) to cause a sudden increase in vit. C of blood of rat. Because glucose as well as acetoacetate, in small doses, accelerated biosynthesis of vit. C, it was thought desirable to see the combined effect of the two in germinating mung bean (*Phaseolus mungo*), as well as in the body of the rat. It has recently been briefly reported(7) that though acetoacetate alone has a retarding effect on biosynthesis of vit. C in the germinating mung bean, acetoacetate and glucose added to the medium in equimolecular proportion cause a gradual rise of vit. C. The condensation product of the two(8) which Gonzales(9) showed to be 2-tetrahydroxy butyl 5-methyl 4-carbethoxy furan (I) sharply raised the vit. C (II) level of the germinating mung bean from the start of the experiment.



(I)



(II)

Experimental. (A) *Biosynthesis in germinating mung bean.* Glucose and acetoacetate (Na salt) separately or together and the condensation product of the two were added to the medium for germination of mung bean. Seeds were first soaked in glass-distilled water for about 3 hours, then immersed in aqueous media containing these different substances, at pH 7.4. For each experiment 500 seeds of almost equal weight were selected. Potassium meta bi-sulphite (300 P.P.m.) was added to all media as recommended by Kendrick and Downer(10). One hundred seeds were taken when required, the testa removed and ex-

TABLE I. Effect of Using Equimolecular Proportions of Different Substances on the Production of Ascorbic Acid During Process of Germination of Legumes.

Substances used in germinating medium	Vit. C (mg)/100 germinated seeds and mean deviation after germination			
	24 hr	48 hr	72 hr	96 hr
None	.91 ± .19	1.81 ± .16	2.36 ± .18	2.08 ± .12
Glucose (a)*	1.05 ± .15	1.94 ± .14	2.48 ± .18	2.13 ± .09
Sodium acetoacetate (b)*	.81 ± .04	.61 ± .03	.50 ± .02	.38 ± .09
Glucose + sodium acetoacetate*	.85 ± .10	1.92 ± .06	2.24 ± .17	2.56 ± .19
Condensation product of (a)* and (b)*	1.31 ± .16	2.67 ± .12	3.64 ± .14	4.07 ± .25

* Added in equimolar amounts; glucose being 100 mg.

TABLE II. Effect of Concentration of Condensation Product on the Vitamin C Synthesis.

Substances in germinating medium	Amt of substance (mg/100 seeds)	Vit. C, mg/100 germinated seeds			
		24 hr	48 hr	72 hr	96 hr
Glucose	100	.79	1.85	2.40	2.16
Condensation product	5	1.28	2.00	2.79	2.79
—	25	1.30	2.60	3.00	4.12
—	100	1.37	3.00	4.08	4.15
—	250	1.42	2.92	4.05	4.15

tracted thrice with a mixture of metaphosphoric acid (3%) and acetic acid (8%) and ascorbic acid estimated according to titrimetric method of Bessey(11). Because this method may lead to erroneous results owing to interference from other reducing compounds, the experiment was repeated and ascorbic acid estimated colorimetrically according to the method of Roe and Kuether (12). In all the above processes of germination the seeds were first washed with .1% mercuric chloride solution, then with sterile water and finally kept in sterile petri dishes with the required amounts of sterile distilled water for ideal germination. The results obtained with the Roe and Kuether's method are shown in Table I.

Some experiments were then done to show whether the condensation product itself inhibits the breakdown of the ascorbic acid. Equal amounts of extracts of the germinated beans were placed in 2 sets of flasks, one set containing the condensation product. Known quantities of ascorbic acid were added to all the flasks and the amounts of ascorbic acid in both the sets were determined at regular intervals. The same degree of breakdown was noticed in both the sets. After it was seen that the concentration of vit. C in the germinated seeds is about 100% higher after 72 hours in the dishes containing the condensation product of glucose and acetoacetate (152 mg per 100 seeds), it was thought desirable to study the effect of the concentration of the product on the amount of vit. C formed. The solubility of the condensation product (I) is about 100 mg in 10 cc of water at about 27°C (room temp.).

For the higher concentration of the condensation product, a suspension of the same was made in water because of its poor solubility. The results are shown in Table II.

Table III shows that in a mixture of glucose and acetoacetate a high proportion of the latter depresses the biosynthesis of vit. C.

In order to see if there was any indication of some chemical reaction between glucose and acetoacetate at pH 7.4 (37°C), experiments were carried out with known amounts of glucose with and without acetoacetate. A substantial decrease in glucose concentration

TABLE III. Effect of Glucose with Low and High Concentration of Sodium Acetoacetate on Vit. C Biosynthesis in Germinating Seeds.

Substances in germinating medium	Amt of substance (mg/100 seeds)	Vit. C, mg/100 germinating seeds		
		24 hr	48 hr	72 hr
Water		.96	1.84	2.47
Glucose	100	.80	2.16	2.67
Glucose & Na acetoacetate	10 } 10 }	.99	2.35	2.88
Glucose & Na acetoacetate	10 } 150 }	.52	.78	1.99

TABLE IV. Biosynthesis of Vit. C in Rats Caused by Intramusc. Inj. of Glucose, Acetoacetate Followed by Its Equimolecular Amount of Glucose, Condensation Product of Glucose and Ethylacetate and Acetoacetate Respectively (3 Rats in Each Series).

Substances inj. (mg/100 g body wt)	Avg values of vit. C/100 cc plasma (mg) 0 hr	1 hr	4 hr
Acetoacetate, 100	1.40 ± .03	1.80 ± .04	.70 ± .02
Glucose, 145.2	1.41 ± .02	1.70 ± .08	1.41 ± .02
Acetoacetate, 100, immediately followed by glucose, 145.2	1.42 ± .02	1.98 ± .18	1.42 ± .05
Condensation product of glucose & ethylacetoacetate, 25 in solution	1.42 ± .02	2.5 ± .05	1.42 ± .02
Ascorbic acid, 16 (equivalent to 25 condensation product)	1.41 ± .02	2.9 ± .18	1.41 ± .02

was observed after 72 hours of incubation in presence of acetoacetate. Experiments on the effect of acetoacetate in germinating medium on utilization of added glucose also indicated great loss of glucose after germination for 24 hours in presence of acetoacetate, provided its quantity did not exceed the equimolecular amount of glucose.

(B) *Biosynthesis of ascorbic acid in the animal system.* It was thought necessary to see how glucose and acetoacetate can influence the synthesis of vit. C in the body of the rat, which is known to synthesize the vitamin. Rats weighing about 180 g were used for the experiment, and the different substances were injected into them intramuscularly. Blood was collected from rats by clipping the tails. 0.7 cc of blood was collected for each estimation, before and after injections, and plasma was obtained after centrifuging. To 0.3 cc of the plasma 2.7 cc of 5% metaphosphoric acid was added for deproteinization. The clear extract obtained after centrifuging was used for the determination of ascorbic acid according to the method of Mindlin and Butler(13) by the use of the photoelectric colorimeter. The results are shown in Table IV.

Discussion. Szeki and Laszlow(14) succeeded in oxidizing the condensation product (I) to 5-formyl furan 3-carboxylic ester, furan 3-5-dicarboxylic acid and furan 1-3-5-tricarboxylic acid respectively. Again, it is quite interesting to note that the work of Muller and Varga(15) on the oxidation of the condensation product (I) with excess of $Pb(oAc)_4$ has definitely revealed a cleavage between the 4-5 C atoms of the original carbon chain of the glucose molecule. Thus the transformation of the condensation product (I) into vit. C (II) may be pictured through the combined process of oxidation and hydration which might be taking place during the course of germination.

Further, the possibility of the conversion of the condensation product to any substance or substances other than ascorbic acid, having the property of reducing the dye, can also be eliminated through the recent findings of Nath and Sahu(16) who, as a result of hydrolytic and oxidative effects of HCl and alkaline H_2O_2 respectively on the condensation product, obtained a number of substances, none of which can reduce the dye. That there is a chemical reaction taking place between glucose and acetoacetate is also indicated by the formation of a yellow color in the reaction mixture. This color formation, observed after 24 hours incubation, deepens in intensity if the period of incubation is further increased to 48, 72, or 96 hours, thus showing that there is more and more of a reaction between the two, glucose and acetoacetate. That the color formation is only due to a reaction between glucose and acetoacetate and not between glucose and acetone, which is formed as a result of hydrolysis of Na acetoacetate, was nicely indicated by a control experiment wherein glucose was incubated with acetone under identical conditions. Further, the findings of Illari(17) are also in accord with our view of a reaction between glucose and acetoacetate. The data indicate that the biosynthesis of ascorbic acid is the result of a reaction between glucose and acetoacetate which in all probability appears to be an enzymatic one.

It is possible that the metabolic relationship between glucose and acetoacetate, which has just been reported by Nath and Sahu(18) to exist in the living body, assumes a much greater significance and importance in both animals and plants.

That this condensation product of glucose and acetoacetate acts as a precursor to ascorbic acid has also been substantiated by *in vivo* studies on rats. Higher values of the vit. C in the plasma of rats have been noticed (Table IV) after one hour of injection of acetoacetate following that of glucose or of the condensation product of the two given in water solution. Further, these data also show the beneficial role of glucose towards the toxic effect of acetoacetate and supports the view of a metabolic relationship between these two compounds(18).

Summary. 1. Though glucose has a slight effect in the process of biosynthesis of ascorbic acid in the germinating mung beans and acetoacetate alone has rather a destructive effect, the product formed by the condensation of the two (2-tetra hydroxy butyl 5-methyl 4-carbethoxy furan) has been found to accelerate the process greatly. 2. Glucose and acetoacetate when added in equimolecular amounts to the germinating beans have also been shown to cause greater biosynthesis of ascorbic acid. 3. The condensation product of glucose and acetoacetate has no inhibitory effect on the loss of ascorbic acid and the probability of this compound, which is also structurally similar to ascorbic acid, acting as a precursor of ascorbic acid has been suggested. 4. Injection of the condensation product of glucose and acetoacetate or of one following the other has been found to raise the ascorbic acid level in the plasma of rats. 5. Glucose has been found to prevent the harmful effect of acetoacetate with respect to depletion of ascorbic acid *in vivo*. 6. Pathways of glucose metabolism not previously recognized have been suggested.

Addendum. After this paper was communicated for publication, a paper on the origin of l-ascorbic acid in the albino rats has appeared from the laboratory of King (19), which gives confirmatory evidence of glucose taking part in the biosynthesis of ascorbic acid.

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