chymal tissues, or may simply mean that there are other mechanisms of removing collagen which presently are not measurable by tissue culture techniques. Grillo separated epithelium from underlying dermis and mesenchymal tissue in healing wounds of guinea pigs. He found that only epithelium produced lysis but that more lysis occurred when epithelium and mesenchymal tissue were cultured together(5).

A significant number of human ailments are characterized by abnormally large amounts of collagenous tissue, and a few ailments are caused by a deficiency of collagen. Discovery of an enzyme in human tissue which has the ability to lyse collagen molecules suggests the feasibility of looking at such conditions from the standpoint of an imbalance between collagen synthesis and breakdown. The relatively crude methods which show that degradation and synthesis are occurring simultaneously, however, may not be sensitive enough to measure minute differences in enzyme kinetics which, over a long period of time, could account for gross tissue abnormalities. Moreover, the problem of knowing precisely when to look for small abnormalities may be critical, as there is a growing body of evidence to indicate that synthesis is not a continuous process. If this is so, the search for dynamic abnormalities will have to be carefully timed or imbalances which are physiological at certain times could be misinterpreted as pathological, and significant imbalances at crucial times could be overlooked because samples were collected at inappropriate intervals.

Summary and conclusions. 1. Collagenolytic activity in normal and pathological human tissue has been demonstrated by culturing tissue specimens on reconstituted bovine collagen substrate. 2. Collagenolysis appears to be associated primarily with epithelium and not with deep connective tissue elements. 3. Alteration of a delicate balance between collagen synthesis and collagen breakdown in remodeling tissues has been hypothesized as the mechanism by which some pathological conditions characterized by abnormal amounts of collagen may be produced.

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Effect of Acetoacetate and β-Hydroxybutyrate on Vitamin B_{12} in Rats. (31703)

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The intermediary compounds involved in fat metabolism, acetoacetate and β-hydroxybutyrate on prolonged administration have been shown to cause increase in blood sugar (1), blood lactic and pyruvic acids(2,3), and blood urea and inorganic phosphate(4). These ketone bodies also showed increased cholesterol/phospholipid ratio(5), disturbances in the balance of hepatic glycogenesis and glycogenolysis(6), decrease in reduced

TABLE I. The Growth of Rats on the Experimental Diets.

Group	Wt gain in 40 days	Wt gain in 90 days	Wt gain in 120 days
	(mean ± S.D.)		
I Control	90 ± 6 (10)*	128 ± 7 (10)	163 ± 5 (10)
II Acetoacetate	78 ± 8 (15)†	100 ± 9 (12)†	115 ± 10 (10)†
III β-hydroxybutyrate	75 ± 6 (15)†	96 ± 8 (12)†	110 ± 9 (9) †
IV Acetoacetate + vitamin B_{12}	86 ± 7 (10)	120 ± 6 (10)	136 ± 11 (9) †
V β-hydroxybutyrate + vitamin B_{12}	90 ± 8 (10)	121 ± 7 (10)	137 ± 9 (10)†

* Figures in parentheses represent No. of animals.
† P less than 0.01 as compared to Group I using Student's "t" test.

glutathione of blood and tissue(7), decreased succinic oxidase activity(8) and disturbances in other enzyme activities(9). All these are the indices of general disturbances of carbohydrate and lipid metabolism caused by ketone bodies.

It is well understood that metabolism is slowed down and changed by deficiency of vitamins. Therefore, any deficiency of vitamins, single or combined, might cause a general disturbance in body metabolism. It was found that ketone bodies cause enormous depletion of thiamine(3), riboflavin, niacin (10) and vitamin C(2) in animals.

Though the role of vit. B_{12} in maintaining —SH groups has not been established at molecular levels, its participation as a reducing agent in the maintainence of —SH groups has been reported by Dickerman *et al* (11). According to them cobamide maintained an essential —SH group on the methyl transferring enzyme in the reduced state in the biosynthesis of methionine.

Since carbohydrate and lipid metabolism are disturbed by the ketone bodies and since vit. B_{12} is essential for the proper metabolism of carbohydrate and lipid by maintaining reduced glutathione and sulphydryl enzymes (12), it was thought worthwhile to ascertain whether substances like acetoacetate and β-hydroxybutyrate have any influence on vit. B_{12} level in blood and tissue *in vivo*.

Methods. Male albino rats weighing about 40-50 g each were reared on the laboratory stock diet as used by Kasbekar *et al*(13), with slight modification, made up of wheat flour 75%, casein 12%, whole milk powder 2%, dried yeast 2%, groundnut oil 4%, vitaminized sesame oil 1%, sodium chloride 2%, calcium carbonate 2%. Water was given *ad libitum*. The rats were divided into 5 groups and were individually caged. Care was taken to see that coprophagy was prevented. Group I animals were fed on stock diet. Group II and III animals were injected intraperitoneally with sodium acetoacetate (500 mg/kg) and sodium β-hydroxybutyrate (250 mg/kg) respectively. Group IV and V animals were injected intraperitoneally with vit. B_{12} (10 μg/rat) on alternate days along with sodium acetoacetate and β-hydroxybutyrate respectively. Blood was withdrawn by venipuncture after ether anaesthesia and immediately citrated on 40th, 90th and 120th day. Sets of animals were sacrificed on 40th, 90th and 120th day. Livers were excised quickly, frozen in cracked ice, dried with filter paper, weighed accurately and homogenized in a previously chilled Potter-Elvehjem glass homogenizer. Vit. B_{12} was determined in liver and blood samples microbiologically by *Euglena gracilis*(14) and *Lactobacillus leichmannii* respectively(15). Soluble sulphydryl compounds were estimated by the nitroprusside method of Grunert and Phillips (16). Total lipid, total protein, RNA and DNA were estimated by the standard procedures(13,17,18,19).

Growth of the rats in terms of gain in body weight at the end of 40, 90, 120 days was recorded.

Results and discussion. It is evident from Table I that continued injections of acetoacetate and β-hydroxybutyrate retard the growth of animals fed stock diet. The loss in body weight was apparent after 40 days of administration of ketone bodies. The loss in body weight of the rats might be a manifestation of a combined vitamin B-complex depletion including vit. B_{12}. A decrease in the

growth retardation afforded by vit. B_{12} in the animals treated with prolonged and continued injections of ketone bodies suggests an increased requirement for this vitamin.

No major changes were observed in the liver vit. B_{12} level up to 90 days of administration of ketone bodies (Table II). After this period, however, there was a marked fall in liver vit. B_{12} level (P less than 0.01). There was a considerable lowering of blood vit. B_{12} level at the end of 120 days (P less than 0.01). In the animals protected by vit. B_{12}, almost normal levels of this vitamin were found in liver and blood. β-hydroxybutyrate showed a greater effect than acetoacetate in the depletion of vit. B_{12}. Thiamine and riboflavin deficiency produces vit. B_{12} deficiency(20,21). Acetoacetate and β-hydroxybutyrate, which have been previously shown to deplete thiamine, riboflavin and vit. C (3,10,2), can therefore cause a lowering of vit. B_{12} content in liver and blood after the animals are depleted of the above vitamins. Similar depletion of the various members of the vit. B complex by injection of ACTH has been reported(22) and it has been shown that administration of acetoacetate(23,24) and β-hydroxybutyrate(25), causes stimulation of the pituitary-adrenal system especially the secretion of ACTH. These findings suggest that the stimulation of the pituitary-adrenal system and increased secretion of ACTH as a result of ketone body injection, cause vit. B_{12} depletion.

TABLE II. Effect of Acetoacetate and β-Hydroxybutyrate on Liver and Blood Vitamin B_{12} Content.

Group		Blood B_{12} (in mμg/ml) (mean ± S.D.)			Liver B_{12} (in mμg/g) (mean ± S.D.)		
		Days of injections					
		40	90	120	40	90	120
I	Control	1.60 ± .1	1.61 ± .15	1.63 ± .2	108 ± 10	110 ± 8.5	108 ± 7
II	Acetoacetate	1.61 ± .08	1.50 ± .1	1.46 ± .15*	104 ± 8	90 ± 7.6*	66 ± 10*
III	β-hydroxybutyrate	1.63 ± .08	1.51 ± .15	1.39 ± .2 *	103 ± 12	88 ± 5.4*	59 ± 9*
IV	Acetoacetate + vitamin B_{12}	1.81 ± .1	1.76 ± .15	1.73 ± .08	120 ± 8	115 ± 5	113 ± 10
V	β-hydroxybutyrate + vitamin B_{12}	1.70 ± .2	1.81 ± .12	1.83 ± .1	115 ± 10	116 ± 9	112 ± 7.5

* P less than 0.01 as compared to Group I using Student's "t" test.

The lowering of liver vit. B_{12} level was closely followed by changes in the sulphydryl reserves of the liver in the experimental animals. The blood sulphydryl level was lowered at the end of 90 days. Vitamin B_{12} protected animals had normal levels of sulphydryl content in liver and blood. It was earlier reported that the ketone bodies were responsible for the lowering of blood GSH(7) and the present work shows that they have a similar effect on liver sulphydryl content.

The depletion of liver vit. B_{12} and impairment in sulphydryl metabolism precedes the general derangement in liver functions as is indicated by Table III. Total lipid content of the liver showed a slight increase after administration of ketone bodies. There was also a lowering of phospholipid, slight but significant decrease in total protein and considerable depletion of RNA (P less than 0.01). The DNA content of the liver was unaltered. The protective action of B_{12} against the ketone bodies is evident in Table III. It afforded protection as seen from restoration of almost all the constituents.

Summary. The effect of acetoacetate and

β-hydroxybutyrate on the growth of rats and vit. B_{12} levels in blood and liver of rats was studied. Continued and prolonged injection of acetoacetate as well as β-hydroxybutyrate caused depression of growth and marked depletion of vit. B_{12} in blood and liver. There was also a significant reduction in liver sulphydryl content and alteration in other liver constituents such as protein, lipid and RNA. Animals treated with vit. B_{12} showed partial growth depression, and normal liver and blood vit. B_{12} content and other constituents in liver in experimental animals. It is suggested that pituitary may be involved in depletion of vit. B_{12} as a result of injection of ketone bodies.

TABLE III. Alterations in Certain Liver Constituents After Continued Injections of Acetoacetate and β-Hydroxybutyrate for 120 Days.

Group	Total lipid	Total protein	Liver sulphydryl	Liver RNA	Liver DNA
	mg/g of fresh liver tissue (mean ± S.D.)				
I Control	23.6 ± 1.1	200 ± 10	1.39 ± .37	7.40 ± .1	3.84 ± .15
II Acetoacetate	30.1 ± 1.3*	181 ± 8*	.96 ± .2 *	4.41 ± .08*	3.79 ± .1
III β-hydroxybutyrate	29.6 ± 2.3*	176 ± 6*	.89 ± .23*	3.30 ± .12*	3.51 ± .12
IV Acetoacetate + vitamin B_{12}	25.3 ± 1.1	192 ± 9	1.34 ± .14	6.58 ± .11	3.71 ± .13
V β-hydroxybutyrate + vitamin B_{12}	24.8 ± 1.25	192 ± 12	1.29 ± .1	5.96 ± .1	3.86 ± .17

* P less than 0.01 as compared to Group I using Student's "t" test.

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