dase by *S. aureus* in a chemically defined medium than in nonsynthetic media demonstrated the necessity for additional nutritional factors for optimal production of this enzyme. Rogers(1) has reported similar results. The same situation has been reported for production of other enzymes such as lecithinase by *Clostridium perfringens*(7). Tyrosine, phenylalanine, and tryptophan were dispensable for optimal growth of *S. aureus*, strain SAB2, in the synthetic medium used. This result agrees with previous work demonstrating that these 3 amino acids are dispensable for growth of a number of strains of staphylococci(3). However, this organism required tryptophan and tyrosine for synthesis of hyaluronidase. Glycine was completely essential for growth. Furthermore, an increase in growth and hyaluronidase production was noted when the synthetic medium was supplemented with either glycyl-L-tryptophan or glycyl-L-tyrosine. This may indicate that the peptides enhance growth and enzyme production by supplying the essential amino acids in a more utilizable form(8).

Summary. *Staphylococcus aureus*, strain SAB2, synthesized hyaluronidase in a chemically defined medium only in the presence of tyrosine and tryptophan, even though these amino acids were not required for optimal growth. Glycine was indispensable for growth of this organism. Supplementation of the synthetic medium with glycyl-L-tyrosine or glycyl-L-tryptophan enhanced both growth and hyaluronidase production.

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Liver Ketogenesis: Role of Glucose-Cyclo-Acetoacetate on Ketogenesis From Fatty Acids and Pyruvate. (23853)

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After having shown(1-7) that acetoacetate plays an important role in development of experimental diabetes, we noticed that compounds formed by condensing acetoacetate with glucose are not only non-diabetogenic but act as an important agent in prevention of various kinds of experimental diabetes induced by alloxan, dehydroascorbic acid and acetoacetate(8-12). Because unlike other diabetogenic substances, acetoacetate is a normal metabolite, it is reasonable to assume that gradual accumulation of acetoacetate is responsible for progressive development of clinical diabetes. Such accumulation may be caused either by excessive hepatic ketogenesis (formation of acetoacetate) or by decreased extra hepatic utilization. But it has been demonstrated by Chaikoff *et al.*(13) that tissues of diabetic animals can oxidize ketone bodies as readily as those of normal animals. Raper and Smith(14) are also of the opinion that ketosis must be due, mainly, to this overproduction of ketone bodies by liver rather than to its underutilization by extra-hepatic tissues. Jowett and Quastel(15) found that liver slices form 10 to 40 times more ketone bodies than other organs and Edson(16) has shown that when butyric and crotonic acids

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(Na salt) are added to liver slices, production of acetoacetate is greatly increased. Fatty acids, when added to liver slices are known to be converted to acetyl COA through B-oxidation(17). Pyruvate is also known to be converted to acetyl COA before it is oxidized in the Krebs' Cycle(18). Acetyl COA, derived either from fatty acids or from pyruvate, undergoes condensation to form acetoacetyl COA, which in turn gives rise to acetoacetate(19). Administration of insulin to diabetic animals has been reported by Stadie *et al.*(20) to check rapid production of ketones by the liver. Because glucose-cyclo-acetoacetate prevents onset of acetoacetate-induced and other experimental diabetes, it has been thought worthwhile to see if this compound or any of its derivatives may be helpful in arresting rapid formation of ketone bodies by liver.

We also studied utilization of pyruvate in presence of glucose-cyclo-acetoacetate and its hydrolyzed product.

Methods. Male albino rats of 150 g on adequate normal diet containing 4% McCollum Davis's Salt Mixture, were used. Rats were fasted 18 hours before sacrifice by decapitation. Liver was quickly transferred to ice cold saline. Slices were cut freehand using razor blade. Tissue preparation and incubation methods were described by Umbreit *et al.*(21) in conventional Warburg Vessels (15 cc) and pure oxygen as gas phase was used. Vessels containing Ringer Phosphate buffer (pH 7.2) total volume of fluid in each vessel 3.2 ml were equilibrated 10 minutes at 37.5°C and readings for oxygen uptake were taken during 2 hour incubation period. Then acetoacetate was estimated by aniline citrace method as described by Edson(16). Pyruvate was estimated by method of Friedman *et al.* (22). Preparation of crystalline condensation product was done according to the method of West(23), modified by Nath *et al.*(24). It was dissolved in 2 cc/g 2N NaOH by heating in boiling water-bath for 2 minutes and thus converted to its sodium salt, and was brought to pH 7.2 with HCl, before use. Condensation product was hydrolyzed in 2N HCl for 10 minutes by keeping in the water-bath. The ether soluble fraction was removed and aqueous solution neutralized with NaOH and adjusted to pH 7.2 prior to use. In control vessels, the solution obtained after neutralizing same amount of NaOH was neutralized with HCl to pH 7.2. This solution will compensate excess of sodium chloride present along with Na salt of condensation product or its hydrolysate.

Results. Table I indicates oxygen uptake and production of acetoacetate by liver slices from fatty acids and B-hydroxybutyrate. When glucose-cyclo-acetoacetate or its hydrolysate was added, the acetoacetate produced was much less than that in the normal. Oxygen consumption was, however, slightly depressed. Fatty acids are, therefore, oxidised by liver in presence of this substance in such a way that excess production of acetoacetate is checked. It is well established that acetoacetate and B-hydroxybutyrate are interconvertible in liver and they are not oxidized to any appreciable extent in that organ. When acetoacetate was added to liver slices, its recovery in presence of glucose-cyclo-acetoacetate was, however, not found to be much affected. So also the conversion of B-hydroxybutyrate to acetoacetate is not appreciably affected by these substances. This indicates that glucose-cyclo-acetoacetate or its hydrolysate has no effect on interconversion of acetoacetate to B-hydroxybutyrate. Therefore, acetoacetate formed in presence of liver may be taken as the index of ketogenesis by liver.

It is evident from data in Table II that utilization of pyruvate, as estimated by its disappearance from the incubation media, was much greater in presence of glucose-cyclo-acetoacetate (hydrolyzed). Oxygen uptake was not found to increase considerably and acetoacetate formed, both endogenous and exogeneous, was checked. It is reasonable to assume, therefore, that extra-pyruvate utilized by liver (Table II) and diaphragm (Table III) in presence of these compounds might be used for lipogenesis or glycogenesis, or may be reduced to lactic acid by tissues. This substance was not found to have any effect on the endogenous formation of pyruvate by liver and diaphragm.

Table IV shows that the depressed utiliza-

TABLE I. Acetoacetate Produced and Oxygen Consumed by Liver Slices in Presence of Fatty Acids. (Medium Ringer phosphate buffer, pH 7.2 Temp., 37.5°C.)

No. of rats	Substrate	Substrate conc.	Glucose-cyclo-acetoacetate .016 M	Glucose-cyclo-acetoacetate (hydrolysed) .016 M	Oxygen uptake per 100 mg wet wt of tissue in 2 hr	Acetoacetate produced* per 100 mg wet wt of tissue in 2 hr
5	Crotonate	Nil			236 ± 23	22 ± 8
		.017 M			404 ± 27	128 ± 20
		"	Added		348 ± 35	80 ± 15
		"		Added	330 ± 20	65 ± 11
9	Butyrate	Nil			230 ± 23	22 ± 8
		.017 M			320 ± 23	88 ± 11
		"	Added		300 ± 30	22 ± 9
		"		Added	290 ± 25	20 ± 8
3	Acetate	Nil			269 ± 23	15 ± 5
		.02 M			304 ± 20	60 ± 16
		"	Added		300 ± 30	32 ± 9
		"		Added	281 ± 20	29 ± 9
3	B-hydroxybutyrate	Nil			260 ± 20	11 ± 2
		.01 M			280 ± 24	148 ± 20
		"	Added		280 ± 25	134 ± 8
		"		Added	285 ± 24	132 ± 7
	Acetoacetate	Nil			260 ± 20	11 ± 2
		.0033 M			232 ± 20	76 ± 11
		"	Added		178 ± 21	86 ± 13
		"		Added	175 ± 18	81 ± 14

* CO_2 in μl produced after decarboxylation of acetoacetate with aniline citrate.

TABLE II. Acetoacetate Produced and Pyruvate Utilized by Normal Rat Liver Slices.

No. of rats	Glucose-cyclo-acetoacetate (hydrolysed) .016 M	Pyruvate .008 M	Glucose .006 M	μl of oxygen uptake per 100 mg wet wt of tissue in 2 hr	Acetoacetate* produced per 100 mg wet wt of tissue in 2 hr	Pyruvate disappeared in γ per 100 mg wet wt of tissue in 2 hr
9				194 ± 14	40 ± 16	325 ± 5
9	Added			232 ± 23	18 ± 1	320 ± 7
12		Added		332 ± 19	100 ± 26	2140 ± 90
12	Added	"		355 ± 25	45 ± 3	2490 ± 80
6		"	Added	201 ± 15	61 ± 5	2000 ±100

* CO_2 in μl produced after decarboxylation of acetoacetate with aniline citrate.

TABLE III. Acetoacetate Produced and Pyruvate Utilized by Normal Rat Diaphragm.

No. of rats	Glucose-cyclo-acetoacetate (hydrolysed) .016 M	Pyruvate .008 M	Glucose .006 M	μl of oxygen uptake per 100 mg wet wt of tissue in 2 hr	Acetoacetate* produced per 100 mg wet wt of tissue in 2 hr	Pyruvate disappeared in γ per 100 mg wet wt of tissue in 2 hr
9				200 ± 19	Negligible	200 ± 10
9	Added			207 ± 17	"	200 ± 10
12		Added		253 ± 23	16 ± 2	891 ± 30
12	Added	"		319 ± 30	14 ± 2	982 ± 45
6		"	Added	216 ± 4	14 ± 2	530 ± 25

* CO_2 in μl produced after decarboxylation of acetoacetate with aniline citrate.

tion of pyruvate by liver slices of alloxan diabetic rats is restored to normal value in presence of glucose-cyclo-acetoacetate (hydrolysate). Villee and Hastings(25) observed less utilization or pyruvate by the diabetic rats' diaphragm, and this was restored to normal level by addition of insulin *in vitro* (5 units/cc). Glucose has not been shown to have any effect on utilization of pyruvate by diaphragm and liver. (Tables, II, III).

Summary. (1) Glucose-cyclo-acetoacetate and its hydrolysate prevented ketogenesis by

TABLE IV. Acetoacetate Produced and Pyruvate Utilized by Liver Slices from Diabetic Rats.†

No. of rats	Glucose-cyclo-acetoacetate (hydrolysed) .016 M	Pyruvate .008 M	μl of oxygen uptake	Acetoacetate* produced	Pyruvate disappeared in γ
			——per 100 mg wet wt of tissue in 2 hr——		
3			128 ± 10	24 ± 3	260 ± 10
3	Added		162 ± 10	15 ± 4	260 ± 11
3		Added	174 ± 11	60 ± 7	1970 ± 30
3	Added	"	139 ± 7	20 ± 10	2155 ± 50

* CO_2 in μl produced after decarboxylation of acetoacetate with aniline citrate.
† Alloxan diabetic rats (2 wk)—urine sugar 3%. Air was used as gas phase in vessels.

liver. (2) These substances cause more utilization of added pyruvate, both by liver slices and diaphragm. (3) They have no effect on interconversion of acetoacetate and B-hydroxybutyrate.

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Harmonic Analysis of the Ballistocardiogram. (23854)

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The two mass analysis upon which modern ballistocardiography is based can only be verified for low frequencies(1,2). Above approximately 15 CPS, platform, trunk and limbs no longer move in unison, and the influence of the coupling between heart and skeleton is markedly increased(3,4). On the other hand, frequencies up to 35 CPS may be required to distinguish sequential physiologic events(5, 6). It is therefore important to determine the ballistocardiographic frequency spectrum undistorted by body resonance. In this report