the urine increased after injection of either compound, the increase being mainly in the urinary BD. However, the total excretion of acetoin and BD in any urine sample never exceeded 0·1 mg., so that the rapid disappearance of these compounds from the circulation could not be attributed to excretion.

The results indicate that there is a rapid metabolism of both acetoin and BD. Acetoin injection produced a marked rise in the blood level of BD, while injection of BD caused only a minor rise in the blood acetoin concentration. At the end of each experiment the BD was still maintained at a level considerably above that present before injection, by an amount representing about 10 % of the total of acetoin or BD injected. The decline from this latter level is slow.

DISCUSSION

When pyruvate is injected in doses large enough to produce a marked increase in blood pyruvate level, a small rise in acetoin production results. This could be due either to a small fraction of the metabolized pyruvate being converted into acetoin and butane-2:3-diol or to rapid metabolism of these compounds when produced. The proportion of pyruvate being converted in this way is unknown. Jagannathan & Schweet (1952) reported that pyruvic oxidase in the absence of oxygen produces acetoin. In the intact animal, reduction of acetoin to butane-2:3-diol occurs in anoxaemia. Massive glucose injections do not influence acetoin production; this is not unexpected since the pyruvate level does not rise significantly. With the use of a more sensitive method for estimating acetoin and butane-2:3-diol it has been demonstrated that some pyruvate is converted into acetoin in the intact animal.

The metabolism of acetoin appears to be quite rapid and is associated with a considerable production of butane-2:3-diol. It is not known whether this is a step in the further metabolism of acetoin or whether it is a side reaction. From tissue dispersion experiments (Dawson & Hullin, 1954) it appears that butane-2:3-diol can be metabolized as rapidly as acetoin. Injections of butane-2:3-diol do not produce a significant increase in acetoin level, however. This may indicate either that the normal route of butane-2:3-diol metabolism does not lie through acetoin, or that its rate of conversion to acetoin is slow compared with the further metabolism of the latter.

SUMMARY

1. The production and utilization of acetoin and butane-2:3-diol has been studied in decerebrated cats.

2. Pyruvate injection in such preparations increased acetoin production. Glucose injection had no such effect.

3. Injection of acetoin and butane-2:3-diol demonstrated that these compounds were rapidly metabolized.

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Metabolism of Acetoin

2. METABOLIC CONVERSIONS OF ACETOIN, PYRUVATE AND ACETATE BY RABBIT-KIDNEY TISSUE DISPERSIONS

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In an unpublished study of the fasting blood levels of acetoin and butane-2:3-diol in normal subjects and in cases of manic-depressive psychosis, we have found that an increase in total acetoin plus butane-2:3-diol level occurred in the depressed phases of the disorder while an increased butane-2:3-diol level associated with a decreased acetoin level was observed during the manic phases. These changes could have been due either to an increased production or to a decreased catabolism of these four-carbon compounds.

From experiments with decerebrated cats, it has also been shown (Dawson & Hullin, 1954) that the acetoin content of the extracellular fluid can be

raised as a result of increased pyruvate metabolism. Enzymic studies on animal tissue and bacterial extracts by Jagannathan & Schweet (1952) and on yeast and wheat germ by Singer & Pensky (1952*a*, *b*) have indicated that an isolated system, pyruvic oxidase, is capable of producing acetoin in the absence of oxygen; with oxygen present, acetate is produced. Green, Westerfeld, Vennesland & Knox (1942) found that 'cyclophorase' preparations produced acetoin from pyruvate as substrate in the absence of a 'sparking' agent, e.g. fumarate.

The rate of disappearance of intravenously injected acetoin in the decerebrated cat is rapid and is associated with a concomitant production of butane-2:3-diol. The latter, however, is more slowly metabolized on injection and there is no associated rise in blood acetoin level (Dawson & Hullin, 1954). This suggests that the rate of acetoin metabolism is faster than the rate of conversion of butane-2:3-diol into acetoin.

The material presented in this paper deals with an investigation of the rate of metabolism of acetoin and related compounds by enzyme systems at two levels of integration, namely whole rabbit-kidney tissue dispersions and the corresponding 'cyclophorase' preparations.

EXPERIMENTAL

Enzyme preparations

Rabbit-kidney tissue dispersion. The kidneys from a 2 to 3 kg. rabbit were removed rapidly after death and exsanguination and disintegrated in a homogenizer (M.S.E.) for 2 min. in 20 ml. of 0·9% KCl.

'*Cyclophorase*' *preparation* R_3K. This was prepared according to the method of Green, Loomis & Auerbach (1948).

Enzymic methods. To investigate the metabolism of acetoin, butane-2:3-diol (BD), diacetyl, acetate and pyruvate by either the whole kidney-tissue dispersion or the 'cyclophorase' preparation R_3K, the following procedure was adopted for each substrate. Into each of three Warburg flasks and two boiling tubes were pipetted: 0·2 ml. 0·1 M-KCl, 0·2 ml. 0·1 M-$MgCl_2$, 0·2 ml. 2×10^{-4} M cytochrome *c*, 0·2 ml. 0·0133 M adenosine triphosphate (ATP), 0·1 ml. 0·01 M sodium fumarate, 0·6 ml. 0·2 M phosphate buffer (pH 7·4) and 0·5 ml. 0·1 M solution of substrate. Tissue dispersion or 'cyclophorase' preparation (1 ml.) was added to each flask and tube immediately after preparation. The flasks and tubes were then shaken at 37° for 15 min.; after this equilibration, the reaction in the tubes was stopped (see below) and the CO_2 production and O_2 utilization in the Warburg flasks during the next 60 min. were determined. The material in the tubes was used to determine the concentration of substrate, acetate, acetoin and BD at the start of the balance experiment. After 1 hr., the reaction in the Warburg flasks was also stopped and the material used to determine the final concentration of substrate and possible metabolic products.

For the estimation of acetoin, BD, diacetyl and pyruvate, the reaction was stopped by the addition of 3 ml. of 10% (w/v) trichloroacetic acid. Acetate was determined on the supernatant solution after treatment of the reaction mixture with 5 ml. of 2% (w/v) $ZnSO_4.7H_2O$ and 0·5 ml. of 0·5 N-NaOH. Preliminary treatment with 1·5 ml. of 20% (w/v) $CuSO_4$ and 1·5 ml. of 10% (w/v) $Ca(OH)_2$ suspension was necessary for the acetoacetate estimation.

These various additions to the tubes and Warburg flasks were made in such a way that, with one tissue dispersion or 'cyclophorase' preparation, we could determine the amounts of acetoin, BD, pyruvate, acetate and acetoacetate both in the initial reaction mixture and after the O_2 and CO_2 estimations had been carried out. CO_2 production was determined by the direct method of Dixon (1943).

Analytical methods

Acetate. The steam-distillation method of McClendon (1944) for volatile fatty acids was used.

Acetoacetate. The method of Edson (1935) was employed in which the acetoacetic acid production was measured by decarboxylation with aniline citrate after removal of any oxaloacetate by incubation of the flask contents at 37° for 20 min. with copper–lime reagent.

Pyruvate. The method of Friedemann & Haugen (1943) was used.

Acetoin and BD. These were estimated by the method of Happold & Spencer (1952).

RESULTS

Kidney tissue dispersions

The rates of oxidation of acetoin, BD, diacetyl, acetate and pyruvate are presented in Figs. 1 and 2. These values are for oxygen uptake less that of the control in absence of substrate; the latter has a high endogenous respiration (400 μl./hr.) partly due to the 1 μmole of fumarate which is added to all flasks. The period of incubation before the respiration was measured was approximately 15 min.

Initially the endogenous respiration of the tissue dispersion is not stimulated by either acetate or pyruvate. On the other hand, acetoin and BD both stimulate the respiration from the beginning, probably because they are oxidized concurrently with the metabolites already present in the system. Diacetyl resembles pyruvate in its failure to increase the initial basal respiration rate.

To test the effect of the basal metabolite level, the tissue dispersions were pre-incubated for 50 min. at 37° before any respiratory measurements were made. The results shown in Figs. 3 and 4 demonstrate that pyruvate and acetate have a greater effect on the basal respiration rate than has acetoin. The endogenous oxygen uptake of the preparation is greatly diminished by this pre-incubation. Butane-2:3-diol and diacetyl are as effective as pyruvate in stimulating respiration. The difference between the shapes of the pyruvate curves in Figs. 3 and 4 is probably due to the higher endogenous metabolite level in the tissue preparation used in Fig. 4 (478 μl./hr.) compared with that used in Fig. 3 (185 μl./hr.). The endogenous metabolite level, is of course, much higher in the preparations shown in

Figs. 1 and 2, but the shape of the curve in Fig. 4 resembles these rather than Fig. 3 where a more complete exhaustion of endogenous metabolites has presumably occurred. Furthermore, when pyruvate and acetoin are both present, it can be demonstrated that no competition occurs, the sum of the separate oxygen consumptions being equal to the oxygen uptake when both are present together.

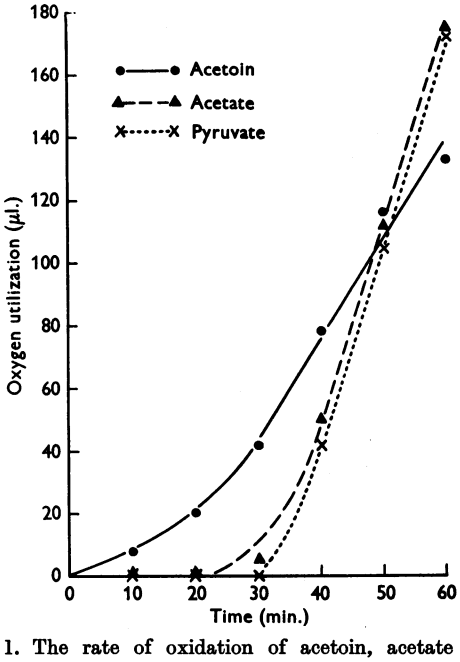

Fig. 1. The rate of oxidation of acetoin, acetate and pyruvate by rabbit-kidney tissue dispersion at 37° after an initial incubation period of 15 min.

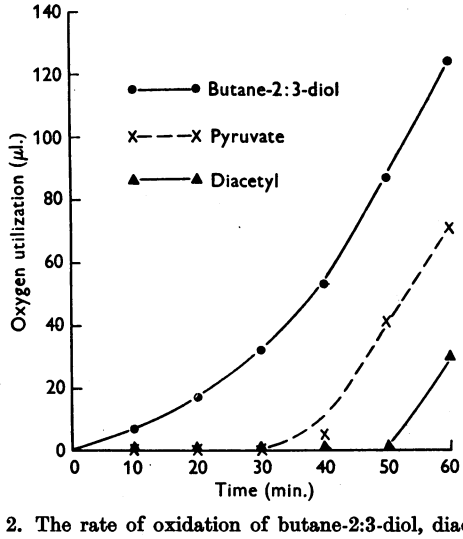

Fig. 2. The rate of oxidation of butane-2:3-diol, diacetyl and pyruvate by rabbit-kidney tissue dispersion at 37° after an initial incubation period of 15 min.

The relationship between oxygen uptake and substrate utilization is presented in Tables 1 and 2. Table 1 indicates the metabolic activity of rabbit-kidney tissue dispersion incubated for 1 hr. at 37° after a short equilibration period of 15 min. The mean $-Q_{O_2}$ values were: for acetoin, 1·6; for butane-2:3-diol, 1·5. Table 2 indicates the metabolic activity after a long equilibration period of 50 min. at 37° during which time the basal metabolite level of the tissue dispersion is greatly reduced.

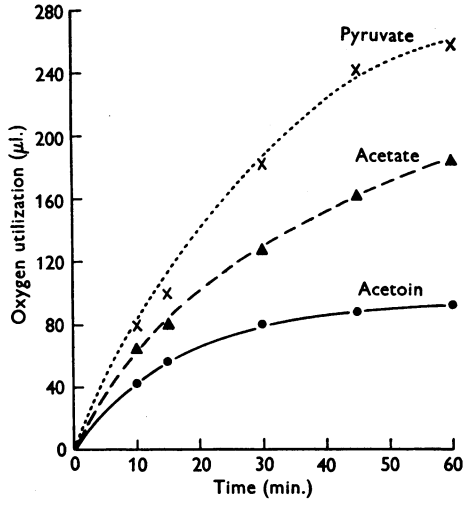

Fig. 3. The rate of oxidation of acetoin, acetate and pyruvate by rabbit-kidney tissue dispersion at 37° after an initial incubation period of 50 min.

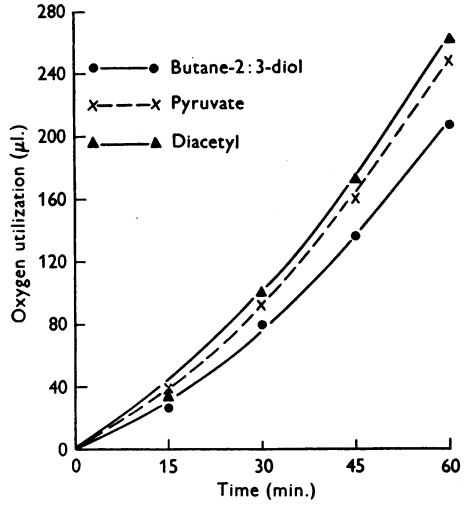

Fig. 4. The rate of oxidation of butane-2:3-diol, diacetyl and pyruvate by rabbit-kidney tissue dispersion at 37° after an initial incubation period of 50 min.

Table 1. *Balance experiments with rabbit-kidney tissue dispersion*

Incubation for 1 hr. at 37° in 0·04 M phosphate buffer, pH 7·4, with various substrates in the medium defined in the Experimental section, with 1 μmole fumarate present. Preliminary equilibration period of 15 min. at 37°. Values are given in μmoles.

	Changes in amounts of metabolites						
Substrate	Substrate	O_2	CO_2	Acetoacetate	Acetate	Acetoin	Butane-2:3-diol
Butane-2:3-diol	−12·4	−5·1	+3·8	+1·2	0	+0·55	—
	−12·9	−5·5	+5·3	+0·9	0	+0·31	—
Pyruvate	−16·0	−3·4	+9·5	+0·9	0	—	+0·5
	−21·0	−2·9	+11·4	+0·6	+3·2	—	+0·15
	−17·6	−7·8	+15·8	+1·1	−1·3	—	+0·1
	−22·5	−4·3	+12·6	+1·2	0	—	+0·1
Acetoin	−8·8	−6·2	+7·6	0	+0·6	—	—
	−6·7	−5·5	+6·0	+1·2	+0·25	—	—
Acetate	−7·6	−7·9	+9·1	+1·3	0	0	0
	−4·9	−5·1	+6·9	+1·3	0	0	0

Table 2. *Balance experiments with rabbit-kidney tissue dispersion after pre-incubation*

The dispersion was pre-incubated with substrate for 1 hr. at 37° before commencing the balance study. Values are given in μmoles.

	Changes in amounts of metabolites						O_2		CO_2	
Substrate	Substrate	O_2	CO_2	Aceto-acetate	Acetate	Acetoin	Found*	Theor-etical†	Found*	Theor-etical†
Butane-2:3-diol	−12·1	−9·2	+8·0	+0·25	0	+1·8	−8·8	−55·5	+8·0	+40·4
Pyruvate	−5·5	−11·1	+16·4	+0·1	−2·7	0	−5·7	−13·3	+10·8	+15·9
	−8·2	−12·3	+19·9	+0·8	−0·2	0	−11·1	−16·5	+17·9	+19·8
Acetoin	−5·4	−4·1	+4·6	+0·4	+0·4	0	−3·5	−23·0	+4·6	+19·2
Acetate	−4·9	−8·2	+8·7	+0·7	—	0	−8·2	−7·4	+8·7	+7·4

* Observed values corrected for acetoacetate, acetate and acetoin production.

† Values calculated for complete oxidation of that portion of substrate not accounted for by production of acetoacetate, acetate or acetoin.

From Table 1 it will be seen that utilization of pyruvate is greater than that of any other of the compounds tested as substrate. All compounds studied were capable of producing acetoacetic acid; pyruvate produced significant amounts of butane-2:3-diol while butane-2:3-diol gave significant amounts of acetoin.

The results in Table 2 show that, with the pre-incubated dispersions, the utilization of pyruvate was greatly diminished whilst the utilization of acetate, acetoin and butane-2:3-diol is not significantly affected. The oxygen uptakes with pyruvate as substrate now have values of 45–60% of those expected for complete oxidation of the pyruvate utilized while the carbon dioxide production values are 70–90% of theoretical. Acetate and, to a lesser extent, pyruvate are metabolized by pre-incubated dispersions in a manner suggesting complete oxidation to CO_2 and water. Acetoin and butane-2:3-diol are still utilized at a rate greater than can be explained on the basis of either complete oxidation or acetate and acetoacetate production.

'*Cyclophorase*' *preparations*

To determine how far the tricarboxylic acid cycle participated in the oxidation of diacetyl, acetoin and butane-2:3-diol, their oxidation by 'cyclophorase' preparations with and without catalytic quantities of added fumarate, was studied. To demonstrate the activity of the 'cyclophorase' preparation, pyruvate oxidation was studied concurrently in a similar manner. The results are shown in Table 3. The mean $-Q_{O_2}$ values for acetoin and butane-2:3-diol were 1·0 and 0·08 in the presence of fumarate after deducting the oxygen consumption of the preparation with fumarate alone as substrate. Comparable values obtained in the same way with the tissue dispersion were: for acetoin, 1·6; for butane-2:3-diol, 1·5. Hence the rate of oxygen consumption of the tissue dispersion with these substrates in the presence of catalytic quantities of fumarate was greater than that of the 'cyclophorase' preparation under similar conditions. When fumarate was omitted from the system, however, the $-Q_{O_2}$ values for acetoin and butane-2:3-diol, respectively, were: for the tissue dispersion, 0·3 and 0·9; for the 'cyclophorase' preparation, 0·7 and 0·0. Thus whilst fumarate is required for the maximum rate of oxidation of acetoin and butane-2:3-diol by tissue dispersions, it does not substantially increase the rate of oxygen uptake by 'cyclophorase' preparations using these substrates.

Table 3. *Oxygen uptake by rabbit-kidney 'cyclophorase' preparations* (R_3K)

Incubation for 1 hr. at 37° with various substrates, with and without the addition of 1 μmole of fumarate. The O_2 uptakes in absence of substrate without added fumarate were always less than 10 μl./hr.

		O_2 uptake (μl./hr.)				
Substrate		Substrate	Substrate +fumarate	Pyruvate	Pyruvate +fumarate	Fumarate
Butane-2:3-diol	(i)	0	18	28	102	16
	(ii)*	0	0	17	81	0
Acetoin		14	19	6	22	8
		20	16	18	38	2
		13	27	18	62	10
		19	39	17	92	10
		17	18	9	27	2
		20	141	76	446	94
		26	100	33	291	56
		23	53	25	127	25
Acetate		288	851	87	412	25
Diacetyl		97	164	51	423	88
		133	187	28	243	34
		176	240	205	486	149

* Same R_3K preparation as (i) but after standing for 3 hr. at 0°.

Table 4. *Acetoacetic acid production by rabbit-kidney 'cyclophorase' preparations*

Experiments carried out at 37° in Warburg flasks containing 1 ml. R_3K suspension, 0·5 ml. 0·1 M substrate, 0·2 ml. 2×10^{-4} M cytochrome *c*, 0·2 ml. 0·1 M-KCl, 0·2 ml. 0·1 M-$MgCl_2$, 0·2 ml. 0·0133 M-ATP and 0·7 ml. 0·2 M phosphate buffer, pH 7·4.

	Acetoacetic acid production in presence of	
Expt. no.	Acetoin (μmoles/hr.)	Pyruvate (μmoles/hr.)
1	1·17	1·23
2	1·15	1·74
3	1·86	1·78
4	0·91	1·36

Table 5. *Balance experiments with rabbit-kidney 'cyclophorase' preparations*

Incubation at 37° for 1 hr. in 0·04 M phosphate buffer, pH 7·4, with acetoin or pyruvate as substrate. Incubation medium as defined in Experimental section except that fumarate (1 μmole) was added only where indicated. Values are given in μmoles.

		Changes in amounts of metabolites				
Expt.	Substrate	Substrate	O_2	CO_2	Acetoacetic acid	Butane-2:3-diol
1	Acetoin	−1·44	−1·57	+0·29	+0·16	—
	Acetoin +fumarate	−2·25	−0·77	+0·37	+0·85	—
	Pyruvate	−2·2	−0·81	+1·59	+0·23	+0·21
	Pyruvate +fumarate	−3·3	−2·36	+4·02	0	+0·18
2	Acetoin	−2·11	−0·84	+0·49	+0·20	—
	Acetoin +fumarate	−3·37	−1·28	+0·88	+0·30	—
	Pyruvate	−1·83	−0·76	+3·12	0	+0·35
	Pyruvate +fumarate	−4·1	−3·64	+7·23	0	+0·90

In view of this lack of effect of added fumarate, we investigated whether the conversion of acetoin into acetoacetic acid could occur. The results in Table 4 demonstrate that acetoin produces as much acetoacetic acid as does pyruvate under the action of 'cyclophorase' preparations and that the amount is not less than that found with whole kidney-tissue dispersions.

Balance experiments where the utilization of pyruvate and acetoin were measured in the presence and absence of fumarate by 'cyclophorase' preparations are presented in Table 5. As will be seen, fumarate stimulates slightly the utilization of acetoin by 'cyclophorase' preparations but neither the O_2 consumption nor CO_2 production would fit the requirements of complete oxidation. As acetate production was not measured, some of the acetoin disappearance may be due to conversion into acetate. The utilization of acetoin is very much smaller than with the tissue dispersions.

Table 6. *Production of butane-2:3-diol from pyruvate by rabbit-kidney tissue dispersions and 'cyclophorase' preparations*

In addition to the enzyme preparation (1 ml.) and 0·5 ml. of 0·1 M pyruvate, the reaction mixture is the same as that given in the Experimental section. Incubation at 37°. The figures are the amounts of BD produced, in μmoles.

Expt. no.	Incubation period (min.) 15	75	135
	Tissue dispersion		
1	—	0·88	0·71
2	—	0·95	0·72
3	0·87	1·37	—
4	0·47	0·62	—
5	0·65	0·76	—
6	1·54	1·66	—
7	0·95	0·91	—
	'Cyclophorase' preparation		
8	1·59	2·37	—
9	1·65	2·18	—
10	2·78	1·88	—
11	1·48	1·45	—
12	1·35	1·45	—

The production of butane-2:3-diol from pyruvate by both rabbit-kidney tissue dispersions and 'cyclophorase' preparations is demonstrated in Table 6. Although much more pyruvate is utilized by dispersions than by 'cyclophorase' preparations, usually more butane-2:3-diol was produced by the latter than by the former.

DISCUSSION

The metabolism of acetoin, butane-2:3-diol and diacetyl by kidney-tissue dispersions is more rapid than by kidney 'cyclophorase' preparations. The rate of disappearance of substrate is increased by the presence of catalytic quantities of fumarate. Fumarate stimulates both the oxygen consumption and carbon dioxide production of the tissue dispersions but the effect on 'cyclophorase' preparations is much less marked.

Kidney-tissue dispersions on ageing at 37° lose some of their ability to remove pyruvate. This ageing process does not, however, affect the rate of removal of acetate, acetoin or butane-2:3-diol. This result may indicate that acetoin and butane-2:3-diol are not oxidized to carbon dioxide and water to any great extent or that the initial step in the production of active two-carbon fragments from pyruvate is the one mainly affected by the ageing and that this step is not required in the oxidation of acetoin and butane-2:3-diol.

It is assumed that acetoacetate production is dependent on the condensation of two active two-carbon fragments. If this mechanism operated for the production of acetoacetate from acetoin and butane-2:3-diol observed with the 'cyclophorase' preparations, it would suggest that two-carbon fragments can be produced from these compounds by 'cyclophorase'. The rate at which this occurs must be slow since fumarate does not markedly stimulate the rate of oxidation although, with acetoin in 'cyclophorase' preparations, it enhances the rate of substrate removal.

In rabbit-kidney tissue dispersions, the rate of acetoin and butane-2:3-diol removal is very much greater than can be accounted for on the basis of complete oxidation or acetate and acetoacetate formation. One possible explanation for this is that these compounds are converted into fat.

Pyruvic oxidase can produce either acetate or acetoin from pyruvic acid (Jagannathan & Schweet, 1952). In both tissue dispersions and 'cyclophorase' preparations a marked production of butane-2:3-diol from pyruvate was noted. From the investigations presented here it appears that acetoin is metabolized largely by a route which might involve fat formation whilst acetate is more completely oxidized. As a working hypothesis it is suggested that the factors affecting fat formation from carbohydrate may act by determining what proportion of the pyruvate produced is converted to acetoin. The levels of butane-2:3-diol production found (Table 6) were apparently the balance state between production and utilization, as they did not always increase with time of incubation of pyruvate with enzyme.

SUMMARY

1. The rate of utilization of acetoin, butane-2:3-diol, acetate and pyruvate by rabbit-kidney tissue dispersions is greater than in the corresponding kidney 'cyclophorase' preparations.

2. Both systems of enzymes can produce acetoacetic acid from acetoin and butane-2:3-diol.

3. The rate of disappearance of acetoin, butane-2:3-diol and pyruvate on incubation at 37° with kidney-tissue dispersions is much greater than can be accounted for by acetoacetate or acetate formation.

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