That an inadequate combustion of carbohydrate is fundamentally responsible for the phenomenon of ketosis is generally accepted. But whether carbohydrate inhibits ketosis by increasing utilization of preformed ketone substances (ketolysis) or by decreasing their formation (antiketogenesis) is still an open question in spite of considerable study. Regarding the utilization of ketone substances, adequate evidence exists that acetoacetic and β-hydroxybutyric acids can be burned by the intact organism, perfused organs, or isolated tissue. On the other hand, such utilization has not been established for acetone. Relatively little is known about the pharmacological action of these substances, inasmuch as their experimental production or clinical occurrence is invariably associated with gross metabolic disturbances.

In the studies of Valdiguie (1) injection of acetone in physiological saline solution in dogs produced, upon analysis of various organs and tissues, a marked rise of β-hydroxybutyric acid. This increase was greatest in the liver and least in the blood. Hahn (2) showed that after the administration of acetone most of it was excreted through the lungs and only a small fraction in the urine. The fact that the respired air was not acetone-free until 24 hours later would indicate slow utilization. Rothkopf (3) showed that in normal subjects 24 per cent of 1 gm. of administered acetone was excreted through the respiratory tract. Caceuri (4) reported that intravenous administration of acetone to rabbits caused a marked increase for several hours of blood acetone, acetoacetic

* A preliminary report was read before the meeting of the American Society of Biological Chemists at New Orleans, March, 1940.
Acetone and Acetoacetic Acid

acid, and especially β-hydroxybutyric acid. A concomitant rise in blood sugar was also found.

The knowledge of acetoacetic acid metabolism is more complete but not without contradictions. Reviews of the findings and conclusions are well summarized in recent literature (5–8). Deuel et al. (9) present evidence based on excretion studies after administration of sodium acetoacetate that would warrant assumption of the ketolytic action of carbohydrate metabolism except for the fact that difficulties are encountered in ruling out endogenous changes. Chaikoff and Soskin (10) found that neither glucose nor insulin affected the rate of disappearance of administered sodium acetoacetate from the blood of the nephrectomized dog. In the depancreatized animal insulin did materially increase this rate but they believed this to be only due to the suppression by insulin of the endogenously produced ketosis, for it did not occur when the animal was eviscerated. Dye and Chidsey (11) also found that injected glucose did not increase the rate of disappearance of administered sodium acetoacetate from the blood of nephrectomized animals.

Friedemann (7) gave sodium acetoacetate intravenously at a constant rate to dogs for 6 to 8 hours and found little or no effect from fasting, insulin, or pancreatectomy. However, in the summary of experiments only one depancreatized dog was reported and the conclusions were based on urinary ketone excretion alone. The significance of urinary excretion can be questioned especially in these experiments, because the animals were described as anuric except for the diuretic effect of the injected salts. These animals apparently were in a state of shock, because artificial heat had to be applied to maintain body temperature and their blood was described as “viscous and dark.” The lack of effect of insulin and pancreatectomy on ketone metabolism of tissue is summarized by Stadie et al. (5).

In our approach to this problem we felt certain advantages could be obtained by intravenous injections at a constant rate of ketone substances into diabetic and non-diabetic human subjects with and without insulin or glucose. These studies can be made without narcosis or operative procedure, under basal conditions, and with a minimal disturbance of the general metabolism. Furthermore, human subjects are more sensitive to ketogenic factors
than the dogs (7) or rats usually used for these investigations, since the latter apparently have a very high comparative tolerance.

Methods

In preliminary investigations it was learned by giving at first small and then increasing amounts that neutral acetoacetate and acetone in saline could be safely administered in effective amounts for blood, urinary, and respiratory studies without any discomfort to the subject. 10 gm. of acetone in 200 cc. of saline or 10 gm. of acetoacetic acid as the neutral sodium salt in water were injected for a period of 2 hours by means of an electrically driven, constant rate pump devised by one of us (12). The volume of fluid injected was sufficiently small to prevent any appreciable disturbance in hemoconcentration. The tests were all started in the morning under fasting conditions and rest in bed.

Acetone, acetoacetic acid, and β-hydroxybutyric acid were determined in blood and urine according to the method of Behre and Benedict (13) and Behre (14). The colorimetric determinations were made by means of a photoelectric colorimeter which added greatly to the convenience and accuracy of the readings. To minimize the spontaneous breakdown of acetoacetic acid to acetone, the blood was precipitated and the filtrate aerated as quickly as possible. To determine acetoacetic acid, the aeration procedure of Folin (15) was modified so as to be carried out in a partial vacuum at a pressure of 20 mm. of Hg. Aeration at atmospheric pressure was less satisfactory, because there was an appreciable breakdown of the acetoacetic acid in the longer time required.

For the determination of β-hydroxybutyric acid, sugar and other color-producing substances were removed from urine by the copper-lime method of Van Slyke (16).

Acetone in the expired air was determined by its absorption in alkaline iodine solution according to the method of Folin (15). Blood sugar was determined by the Shaffer-Hartmann method (17).

Results

Expired Acetone—During and after a 2 hour period of injection of acetone the air in the room acquired a distinct odor similar to that found in diabetic ketosis. The sweet or fruity odor of dia-
betic coma was, however, absent. The question naturally arose as to how much of the injected acetone was lost through the expired air, since it had been reported (2) that as much as 24 per cent of the total administered could be thus lost. Table I shows the amounts of acetone expired at the end of the injections and 2 hours later in one normal and five diabetic patients. Actually the exhaled air was analyzed for acetone for 10 minute periods only, but calculated on an hourly basis. Evidently only a small amount of acetone was lost through the respiratory tract. An average for the six subjects of only 22.3 mg. of acetone per hour was found at the end of the injection and 11.3 mg. per hour 2 hours later. Even if the estimated loss for the next 10 hours had continued at the same rate, the amount (113 mg.) would not have been appreciable compared to the 10 gm. injected. Consequently in view of the amount given and the slow injection we believe that the respiratory loss is not significant from the standpoint of the total balance.

Acetone. Blood Values—Fig. 1 shows the average results of changes in blood acetone, acetoacetic acid, and β-hydroxybutyric acid in twelve normal subjects during and after a 2 hour injection of acetone. The striking feature of the blood acetone is its inappreciable drop for 4 hours after the injection was ended. A significant rise of acetoacetic acid occurs and this rise is also maintained, while β-hydroxybutyric acid values are not affected. The

<table>
<thead>
<tr>
<th>Subject</th>
<th>End of injection</th>
<th>2 hrs. later</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg. per hr.</td>
<td>mg. per hr.</td>
</tr>
<tr>
<td>U. J. Normal</td>
<td>32.4</td>
<td>12.8</td>
</tr>
<tr>
<td>M. C. Mild controlled diabetes</td>
<td>13.2</td>
<td>7.8</td>
</tr>
<tr>
<td>C. M. Severe controlled diabetes</td>
<td>17.4</td>
<td>10.2</td>
</tr>
<tr>
<td>H. S. Mild uncontrolled &quot;</td>
<td>27.4</td>
<td>12.6</td>
</tr>
<tr>
<td>J. K. &quot; &quot; &quot;</td>
<td>18.6</td>
<td>11.4</td>
</tr>
<tr>
<td>A. H. Severe partially controlled diabetes</td>
<td>24.6</td>
<td>13.2</td>
</tr>
<tr>
<td>Average</td>
<td>22.3</td>
<td>11.3</td>
</tr>
</tbody>
</table>
rise in $\beta$-hydroxybutyric acid reported in dogs (4) after acetone injection may be explained by the relatively larger amounts given and the more rapid injection.

![Graph showing changes in blood ketones](image1)

**Fig. 1.** Average results of changes in blood ketones in twelve normal subjects during and after a 2 hour injection of acetone.

![Graph showing fate of injected chemicals](image2)

**Fig. 2.** Fate of injected acetone, acetoacetic acid, pyruvic acid, and lactic acid in blood. 10 gm. of each were injected during 2 hours.

In Fig. 2 the slow or inappreciable disappearance of acetone shown in Fig. 1 is compared with the behavior of three other intermediary metabolic products; namely, acetoacetic, pyruvic, and lactic acids injected in the same amounts and at the same rate. There was not a sufficient variation in the urinary excretion of
these substances substantially to alter the blood values. It is of interest to note the rapid disappearance of the carbohydrate intermediary products, pyruvic and lactic acids, as compared with the much slower rates of the fat breakdown products, acetone and acetoacetic acid.

Fig. 3 illustrates the insignificant effect on the blood acetone values during and after injection of 100 gm. of glucose administered simultaneously with the acetone to seven normal fasting subjects.

In Fig. 4 the effect of acetone alone is compared with that of acetone plus insulin. 20 units of insulin were given \( \frac{1}{2} \) hour before the injection was started, and 20 units were added to the acetone-saline solution directly and injected over a period of 2 hours. No appreciable influence of insulin on the blood acetone was noted in seven normal subjects. Since the glucose and insulin additions to the acetone were given to the same persons in whom acetone control injections had already been made, the comparisons are more valid than comparisons of values from different subjects would be.

In Fig. 5 the blood acetone values of a group of non-diabetic subjects are compared with those of diabetic subjects during and after acetone injection. The latter group was only partially controlled, so that the blood ketone substances were just beginning...
to be elevated. In this state the body was apparently burning
the maximum of fat without undue ketone accumulation; that is,

![Graph 4](image)

**Fig. 4.** Effect of a 2 hour injection of acetone alone and acetone plus insulin on blood acetone of normal subjects.

![Graph 5](image)

**Fig. 5.** Blood acetone values of diabetic and non-diabetic subjects after a 2 hour acetone injection.

it had exhausted its antiketogenic reserve. If the beginning ac-
cumulation of ketones was due to impaired ketolysis, then the
further addition of administered ketones should reflect such im-
paired utilization. The average acetone curve for twelve such
diabetic subjects differs little from that of a group of nineteen non-diabetic persons. The average value for the diabetic subjects is somewhat higher at the end of injection than is the value for the non-diabetic subjects but this factor is offset by the more rapid drop of the curve.

Table II

Average Extra Urinary Excretion of Ketone Substances by Nine Non-Diabetic Subjects for 6 Hour Period Following Beginning of Injection of 10 Gm. of Acetone

<table>
<thead>
<tr>
<th></th>
<th>Acetone excreted</th>
<th>Acetoacetic acid</th>
<th>Total ketone substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone alone</td>
<td>90</td>
<td>23</td>
<td>113</td>
</tr>
<tr>
<td>&quot; + 40 units insulin.</td>
<td>76</td>
<td>31</td>
<td>107</td>
</tr>
<tr>
<td>&quot; + 100 gm. glucose intravenously</td>
<td>111</td>
<td>24</td>
<td>135</td>
</tr>
</tbody>
</table>

Fig. 6. Effect of 2 hour injection of 10 gm. of acetoacetate on blood ketone values of six diabetic subjects with and without insulin.

Table II shows the average urinary excretion of extra ketones during and after injection of 10 gm. of acetone for a 6 hour period. The fasting ketonuria values were subtracted from the total 6 hour excretion, so as to give the extra excretion due to the injection. No gross effects of insulin or glucose were noted. The blood values for Table II are given in Figs. 3 and 4.
It is believed that these experiments lend no support to the hypothesis that the ability of the diabetic patient to destroy acetone is impaired during the early stages of ketosis.

**Fig. 7.** Total blood ketone and acetoacetic acid values of thirteen diabetic and three normal subjects after a 2 hour injection of 10 gm. of acetoacetate.

**Fig. 8.** Blood β-hydroxybutyric acid and acetone values of thirteen diabetic and three normal subjects after a 2 hour injection of 10 gm. of acetoacetate. The dashed curves are for non-diabetic subjects and the continuous curves for diabetic subjects.
Acetoacetic Acid. Blood Changes—The blood ketone values after the injection of 10 gm. of acetoacetic acid neutralized to pH 7.0 with sodium hydroxide are shown in Figs. 6 to 8. Fig. 6 gives the comparative values for six fasting diabetic subjects bordering on ketosis, without insulin, and these same six subjects with sufficient insulin (average 30 units during fasting, 15 units ½ hour before and 15 units in intravenous injection for 2 hours) to bring the fasting blood sugar to normal or what subnormal (100 to 68 mg. per 100 cc.). That an induced acetoacetic acid ketosis produced a rise in all the blood ketone substances is readily apparent. Such a ketosis decreased rapidly during the 2 hours after cessation of injection but still persisted 4 hours later. The effect of insulin, however, on total ketone substances, \(\beta\)-hydroxybutyric acid, ace-

TABLE III

Average Extra Urinary Excretion of Ketone Substances for 6 Hour Period Following Beginning of Injection of 10 Gm. of Acetoacetic Acid

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Acetone excreted</th>
<th>Acetoacetic acid</th>
<th>(\beta)-Hydroxybutyric acid</th>
<th>Total ketone substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 diabetic</td>
<td>81</td>
<td>513</td>
<td>927</td>
<td>1521</td>
</tr>
<tr>
<td>3 normal</td>
<td>13</td>
<td>549</td>
<td>1020</td>
<td>1582</td>
</tr>
<tr>
<td>6 diabetic</td>
<td>71</td>
<td>457</td>
<td>871</td>
<td>1399</td>
</tr>
<tr>
<td>Same diabetics with insulin</td>
<td>64</td>
<td>406</td>
<td>610</td>
<td>1079</td>
</tr>
</tbody>
</table>

toacetic acid, and acetone values was less than the experimental error and can be assumed to be negligible.

Figs. 7 and 8 show the blood ketone values in thirteen diabetic subjects with mild ketosis as compared with three normal persons after the injection of 10 gm. of acetoacetic acid over a period of 2 hours at a constant rate. Fig. 7 shows that the induced changes in total ketone and acetoacetic acid values are practically the same in the two groups. Similarly, the values for \(\beta\)-hydroxybutyric acid and acetone as shown in Fig. 8 for these same groups did not differ significantly.

Urine Changes—Table III shows that the urinary excretion of extra ketones over a 6 hour period after the injection of 10 gm. of acetoacetic acid did not differ greatly in non-diabetic persons and diabetic patients, either uncontrolled or partially or com-
pletely controlled. The blood values for this group are shown in Figs. 6, 7, and 8.

DISCUSSION

We believe that our data on the utilization of ketones in exogenous ketosis by injection at a constant rate in human subjects gives support neither to the hypothesis that in diabetic ketosis a state of impaired ketolysis exists nor to the belief that insulin enhances ketolysis. These findings support those of Chaikoff, Friedemann, Mirsky (18), Dye, and others working with animals. It is probable, however, that the relatively increased susceptibility of man to ketosis as compared with rats or dogs as well as the absence of anesthesia and surgical procedures makes the constant rate injection studies on human subjects recorded here less open to question.

In regard to acetone the question of whether acetolysis is enhanced by insulin or impaired by diabetes must be paired with one as to whether acetone destruction occurs in the body at all even under normal conditions. The slow or inappreciable decrease of acetone in the blood for 4 hours after cessation of its injection even in the normal person suggests very poor or negligible utilization. However, that acetone does disappear from the blood is shown by nearly normal values in 24 hours, but the loss through expired air (average of only 11.3 mg. per hour 2 hours after injection) and the urinary loss (average of 90 mg. for the 6 hours during and after injection) even if considered as continuing at their maximum could not account for the 10 gm. injected. This creates a paradoxical situation which might be explained by the assumption that acetone is destroyed but the maintenance of its high level in blood at first is due to some induced derangement of acetone metabolism such as increased production or impaired utilization. Some basis for such a view can be obtained from Figs. 1 and 2. Fig. 1 portrays the rise in blood acetoacetic acid after acetone injection, reaching a height of nearly 7 mg. per cent at the end of injection and then maintaining an elevation that parallels the sustained higher acetone values. This relationship would at first suggest a definite and continued formation of acetoacetic acid from acetone but Fig. 2 shows that blood acetoacetic acid after injection of 10 gm. of acetoacetic acid goes no higher than the
acetooacetic acid level after acetone injection and quickly drops off after cessation of injection, thus showing rapid utilization. Consequently, if all the 10 gm. of acetone injected were converted to acetooacetic acid, the disappearance of the latter should be at least as rapid as when the same amount of acetooacetic acid is injected. That this is not true indicates the association with the high blood acetone values of either an impairment of proper utilization of acetooacetic acid or an increase in its production. If the older view of ketogenesis is considered, \( \beta \)-hydroxybutyric acid \( \rightarrow \) acetooacetic acid \( \rightarrow \) acetone, then the accumulation of acetone could readily cause an increase in acetooacetic acid formation.

Our failure to obtain a rise in \( \beta \)-hydroxybutyric acid during or after acetone injections as reported in animal experiments (4) is probably due to the relatively smaller amounts given by us.

That the associated rises of \( \beta \)-hydroxybutyric acid and acetone in acetooacetic acid injection are uninfluenced by insulin is shown in Fig. 6. Furthermore, these changes in diabetes do not differ appreciably from those in normal persons (Fig. 8). Friedemann (7) had already found a similar relationship for \( \beta \)-hydroxybutyric acid after acetooacetic acid injections in dogs.

Our experiments showing the rapidity of disappearance of acetooacetic acid and \( \beta \)-hydroxybutyric acid from the blood stream support the findings already quoted in the literature (7, 10). However, the rates of their disappearance are slow compared with those of pyruvic or lactic acids (Fig. 2). The ability of the body to burn acetone, acetooacetic acid, and \( \beta \)-hydroxybutyric acid must be materially below 5 gm. per hour, for when acetone and acetooacetic acid are given at this rate there is a marked accumulation in the blood stream and with acetooacetic acid there is an accumulation of \( \beta \)-hydroxybutyric acid as well. Since, according to the Knoop \( \beta \) oxidation theory, 1 molecule of acetooacetic acid is formed from 1 molecule of fatty acid, 5 gm. of the former could represent 13.8 gm. of oleic acid or approximately 15.3 gm. of fat per hour, 368 gm. per 24 hours, or a maximum of 3300 calories. From another standpoint, calculating from Fig. 7 that it takes about 3½ hours for the blood ketones to return to normal, calculated at maximum rate, after the administration of 10 gm. of acetooacetic acid and allowing 1.5 gm. excretion (Table III), one obtains a basal utilization rate of 8.5 gm. for 3½ hours or 58 gm. for 24 hours. Calculated as oleic acid this would represent 160.5 gm. and as
glyceride approximately 183 gm. or 1650 calories. The average maintenance diet of the subjects studied was about 2200 calories per 24 hours. The tests were made, however, under basal conditions representing about 1300 calories for 24 hours.

It is thus seen that based on these approximate calculations it is probable that the rate of utilization of acetoacetic acid is sufficiently rapid so that no ketosis would occur if no more than 1650 calories of fat were burned but any additional fat combustion would result in accumulation of acetoacetic acid.

If, however, the β oxidation theory is not accepted and the multiple alternate oxidation of fatty acid hypothesis (19, 6, 8) is considered, then 4 molecules of ketones could be formed from a 16 carbon chain fatty acid. Thus the weight ratio of 1 molecule of palmitic acid to 4 of ketone would be 256:416 or 1:1.62. The 58 gm. of acetoacetic acid burned in the above assumption from our values would represent on this basis 35.2 gm. of fatty acid or 39.2 gm. of glyceride or 353 calories for 24 hours. This obviously is far below the ordinary level of fat combustion and this theory would not permit the hypothesis that the ketones represent the only route of fatty acid degradation. Consequently the multiple alternate oxidation hypothesis of fatty acid oxidation in the liver can be reconciled with our data only if the assumption is made that a considerable part of fat metabolism must represent direct utilization in the muscle.

Since impaired ketolysis can, from other investigations and ours, largely be discounted as a cause of ketosis, it must be assumed that increased ketogenesis is primarily responsible for the accumulation of these metabolites. The question then presents itself as to whether the ketones accumulate because they are abnormal substances formed owing to faulty metabolism or whether they are normal intermediary substances produced in such excessive amounts owing to greatly increased fat metabolism. We believe that the latter is the case, although conclusive proof is lacking. This view would completely eliminate carbohydrates as a factor in ketosis except in so far as they may spare fat and protein metabolism.

Symptoms of Induced Ketosis—Schneider and Droller (20) have compared the results produced by hydrochloric acid injection in rabbits with those produced by acetoacetate and concluded that the intoxication effects or coma was due to the specific effect of
Acetone and Acetoacetic Acid

the acetoacetate ion and not due to the acidosis. In our studies of acetoacetic acid injections no symptoms were ever observed, probably owing to the fact that a sufficient amount was not given. With acetone, however, a slight drop in blood pressure, both systolic and diastolic, and a slight transient drowsiness were frequently observed. The anesthetic action of acetone is of course well known.

Effect of Ketosis on Blood Sugar—The relative refractiveness of the blood sugar to insulin during diabetic ketosis is commonly observed. Caccuri (4) has reported an increase in the blood sugar of rabbits after the intravenous injection of ketones. Sugar was determined on all our blood specimens analyzed for ketones after the injection of both acetone and acetoacetic acid and at no time was any change in blood sugar values observed beyond the normal range of variation.

SUMMARY

Acetone and acetoacetic, lactic, and pyruvic acids can safely be injected at the rate of 5 gm. per hour for 2 hours in human subjects without any symptomatic effects except in the case of acetone with which a slight drowsiness may be obtained.

If the maximum increase of the injected metabolites in the blood is taken as being inversely proportional to their utilization, the approximate values are as follows: lactic acid 1, pyruvic acid 3, acetoacetic acid 18, and acetone 30.

The injection of acetone results in a marked rise in blood acetone, and this rise is maintained for a considerable time after the cessation of injection. With the rise in blood acetone there is a concomitant rise of acetoacetic acid but with the amount of acetone we injected no rise in β-hydroxybutyric acid was noted.

Glucose or insulin injections with acetone had no appreciable effect on the increase of acetone or acetoacetic acid during injection nor any effect on the rate of decrease in the blood after cessation of injection. This lack of effect of increased carbohydrate metabolism was also true in regard to the urinary excretion of these ketones.

Acetoacetic acid injection was associated with a rise of this substance in the blood but acetone and β-hydroxybutyric acid increased as well. The control of diabetes with insulin did not appreciably alter the blood ketone values after acetoacetate in-
jections from the values obtained when these same subjects were uncontrolled and in a state of mild ketosis. The increase in acetone and β-hydroxybutyric acid after acetoacetate injections appeared not to be influenced by the control of endogenous ketosis in diabetes with insulin.

The injection of acetone or acetoacetic acid did not appreciably affect the fasting blood sugar.

The rate of acetone breakdown as judged from blood levels and urinary excretion appeared so slow that any large part of normal fatty acid metabolism could not conceivably pass through the acetone stage. On the other hand acetoacetic acid breakdown appears sufficiently rapid for a normal fatty acid route if the β oxidation theory is held valid but only for the multiple alternate oxidation theory of fatty acids if the assumption is made that the greater part of fat metabolism proceeds directly in the muscle.

We believe that our experiments give further and conclusive evidence that carbohydrate metabolism has no ketolytic effect, and that its effect on ketosis is purely antiketogenic.

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