obvious advantages of simplicity and rapidity, electrophoresis on filter paper may be quantitatively more accurate in the analysis of pathological sera containing high proportions of lipid or carbohydrate.

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REFERENCES

Armstrong, S. H. jun. (1950). *Research in Medical Science.* New York: Macmillan Co.
Cremer, H. D. & Tiselius, A. (1950). *Biochem. Z.* **320**, 273.
Crook, E. M., Harris, H. & Warren, F. L. (1952). *Biochem. J.* **51**, xxvi.
Durrum, E. L. (1950). *J. Amer. chem. Soc.* **72**, 2943.
Flynn, F. V. & de Mayo, P. (1951). *Lancet*, **2**, 235.
Grassman, W., Hannig, K. & Knedel, M. (1951). *Deutsch. med. Wschr.* **76**, 333.
Kunkel, H. G. & Tiselius, A. (1951). *J. gen. Physiol.* **35**, 89.
Longsworth, L. G., Shedlovsky, T. & MacInnes, D. A. (1939). *J. exp. Med.* **70**, 399.
Morrison, P. R., Edsall, J. T. & Miller, S. G. (1948). *J. Amer. chem. Soc.* **70**, 3103.
Slater, R. J. & Kunkel, H. G. (1953). *J. Lab. clin. Med.* **41**, 619.
Tiselius, A. (1937). *Trans. Faraday Soc.* **33**, 524.
Turba, H. von & Enenkel, H. J. (1950). *Naturwissenschaften*, **37**, 93.
Wolfson, W. Q., Cohn, C., Calvery, F. & Ichiba, F. (1948). *Amer. J. clin. Path.* **18**, 723.

Time-Course of Injected Acetate in Normal and Depancreatized Dogs

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Acetic acid plays a very important role in intermediary metabolism both as degradation product of large molecules and as building block in numerous synthetic reactions.

It has been shown, mainly with the aid of isotopes, that acetic acid takes part in the syntheses of acetoacetate (Swendseid, Barnes, Hemingway & Nier, 1942), cholesterol (Bloch & Rittenberg, 1942), higher fatty acids (Rittenberg & Bloch, 1944, 1945), citrate (Stern & Ochoa, 1949), etc. Owing to the lack of specific chemical methods for the estimation of acetic acid, few studies of the metabolism of this compound have been made in which the amount of acetic acid metabolized was determined directly.

Recently Ciaranfi & Fonnesu (1952) have described a photometric micromethod for the estimation of acetic acid in blood and tissues and this procedure is suitable for such a type of investigation. With this method it has been shown that acetate is a normal constituent of blood and organs (Ciaranfi & Fonnesu, 1952), of lymph (Fonnesu & Ciaranfi, 1952) and of bile (Fonnesu, 1953*a*). Blood acetate originates either from the tissues or, by absorption, from the intestinal lumen. Skeletal muscle rapidly utilizes acetate conveyed by the blood, since the concentration of acetate in the arterial blood is greater than that in the veins draining the limbs (Fonnesu, 1951, 1953*a*; Fonnesu & Ciaranfi, 1952).

The concentration of acetate in the blood and tissues is relatively low probably because of the rapid turnover of the compound. Experiments with labelled acetate have shown that considerable amounts of acetate are transformed into other compounds and oxidized to carbon dioxide by the intact animal (Buchanan, Hastings & Nesbett, 1943; Lifson, Lorber, Sakami & Wood, 1948; Gould, Sinex, Rosenberg, Solomon & Hastings, 1949; Villee & Hastings, 1949; Pihl, Bloch & Anker, 1950; Hevesy, Ruyssen & Beeckmans, 1951; Coniglio, Anderson & Robinson, 1952; Hutchens & van Bruggen, 1952; van Bruggen, Claycomb, Hutchens & West, 1952). Significant quantities of acetate can also be oxidized by the eviscerated animal (Pihl *et al.* 1950; Wick & Drury, 1952). It has been observed that at least two extrahepatic tissues, that is, the heart (Pearson, Hastings & Bunting, 1949) and the diaphragm (Villee & Hastings, 1949) are capable of oxidizing acetate to carbon dioxide *in vitro*.

However, free acetate is known to be a metabolically inert substance and in order to react it requires activation. The work of Lynen & Reichert (1951) and of Lynen, Reichert & Rueff (1951) made it clear that 'active acetate' is in fact acetyl coenzyme A (Ac ~ CoA).

It is probable that free acetate is first converted into Ac ~ CoA. If this is true, the inhibition of such

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a reaction should lead to the accumulation of acetic acid. Since the concentration of acetate in the urine of human diabetics is greater than that in the urine of normal people (Caselli & Ciaranfi, 1946), it is reasonable to suppose that such a hyperacetaturia may be the consequence of a reduced acetate utilization by the diabetic body.

In the present work we describe experiments on the utilization of acetate by normal and depancreatized dogs, as indicated by the rate of disappearance of injected acetate from the blood stream.

MATERIAL AND METHODS

Normal adult dogs, weighing between 15 and 20 kg., were used. Pancreatectomy was performed by the technique of Haberland (1934) under Pentothal:ether anaesthesia. The depancreatized animals were subjected to the experiment not less than 2 or 3 days after the removal of the pancreas and during this time received neither insulin nor any other treatment. All the animals were fasted for 12 hr. before the experiment.

Normal and depancreatized dogs were given an intracardiac injection of sodium acetate:acetic acid mixture, pH 7·4, containing 25% (w/v) of acetic acid. The amounts injected, expressed as acetic acid, were as follows: 50, 100, 150, 250 mg./kg. body weight. The injection time was always 1 min. Before and at various intervals after the injection, arterial blood samples were taken and the following compounds estimated: acetic acid (Ciaranfi & Fonnesu, 1952), ketone bodies (Weichselbaum & Somogyi, 1941), glucose (Folin & Wu, 1919), cholesterol (Bloor, 1916) and serum phosphate (Fiske & Subbarow, 1925). At the time of the acetic acid estimation, the coloured samples were centrifuged before the photometric reading. A Coleman Spectrophotometer, model S. 14, was used.

Immediately before the acetate was injected, the bladder was emptied by catheter; 30 min. after the injection the bladder was again emptied. The acetic acid concentration in both samples of urine was then determined after Fonnesu (1953*b*). Glucose was estimated in the urine by the Fehling reagent (Bertho & Grassmann, 1936).

RESULTS

Table 1 gives representative values for glucose, ketone bodies and acetate in the blood and for glucose in the urine of both normal and depancreatized dogs before the acetate injection. It will be seen that the blood acetate level is higher in the diabetic than in the normal dogs.

Fig. 1 shows the rates at which different amounts of acetate are removed from the blood of normal and

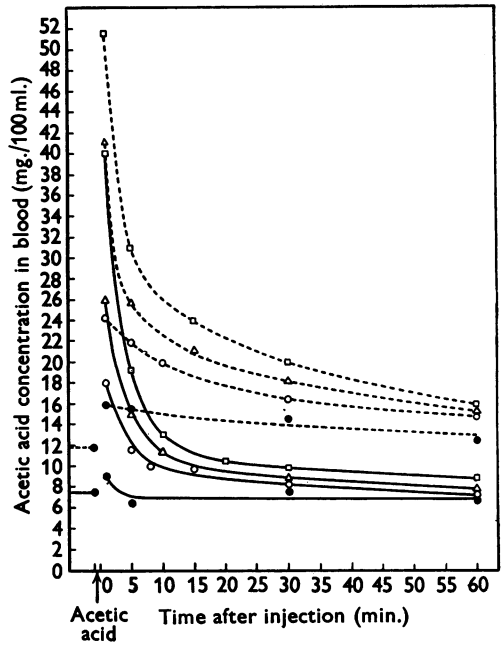

Fig. 1. Time-course of injected acetate in normal (——) and depancreatized (- - -) dogs. The amounts injected, expressed as mg. acetic acid/kg. body weight, were: 50 (●); 100 (○); 150 (△); 250 (□). Each point represents the average of at least three experiments.

Table 1. *Experimental data for normal and depancreatized dogs before the acetate injection*

Type of animal	Arterial blood: Glucose (mg./100 ml.)	Arterial blood: Total ketone bodies (expressed as mg. β-hydroxybutyric acid/100 ml.)	Arterial blood: Acetic acid (mg./100 ml.)	Urine: Glucose excreted (g./l.)
Normal				
Mean ± S.E.	108 ± 2·05	0·94 ± 0·07	7·0 ± 0·41	0
No. of observations	7	7	7	7
Range	100–112	0·69–1·21	5·0–8·0	0·0–0·0
Depancreatized				
Mean ± S.E.	340 ± 24·48	10·98 ± 1·00	11·2 ± 1·18	28·8 ± 4·36
No. of observations	6	6	6	6
Range	280–435	7·9–13·8	8·2–15·4	12–40

Table 2. *Acetic acid concentration in urine (mg./100 ml.) of normal and depancreatized dogs before acetate injection*

	Normal	Depancreatized
Mean ± S.E.	4·6 ± 0·92	9·8 ± 0·62
No. of observations	6	4
Range	2·8–8·0	8·5–11·3

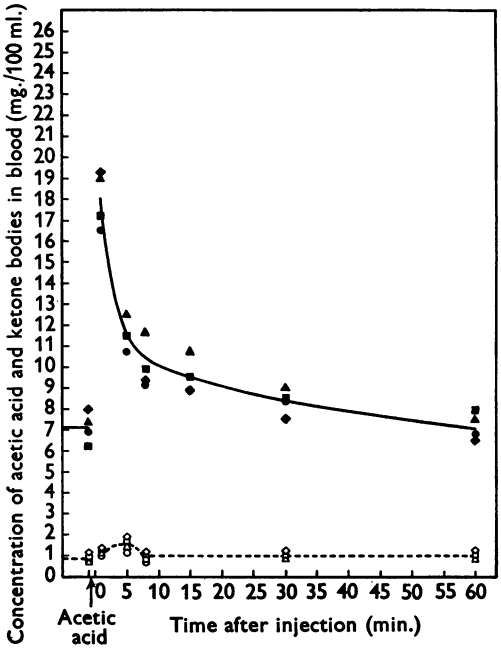

Fig. 2. Relationship between the acetate-disappearance (——) and the formation of ketone bodies (- - - -) in normal dogs. The amount of acetic acid injected was 100 mg./kg. body weight. Points represent values for single animals.

depancreatized dogs. Injected acetate disappears very rapidly from the blood at a rate which is related to the initial blood concentration. Acetate disappears more rapidly from the blood of normal dogs than from the blood of diabetic animals. Taking the half-time as an index of the phenomenon it is observed that in the diabetic animals such a value is 2–6 times greater than normal.

Table 2 shows that the concentration of acetic acid in the urine of diabetic dogs is higher than that in normal animals even before acetate injection.

It should be noted that 30 min. after the injection of acetate the amount of urine found in the bladder, which had been emptied before the injection, was only a few ml. Since the concentration of acetate in these samples was slightly higher than that found in the urine before the injection, the amount of injected acetate eliminated by way of the kidneys (0·2–3 % of the amount injected) may be neglected.

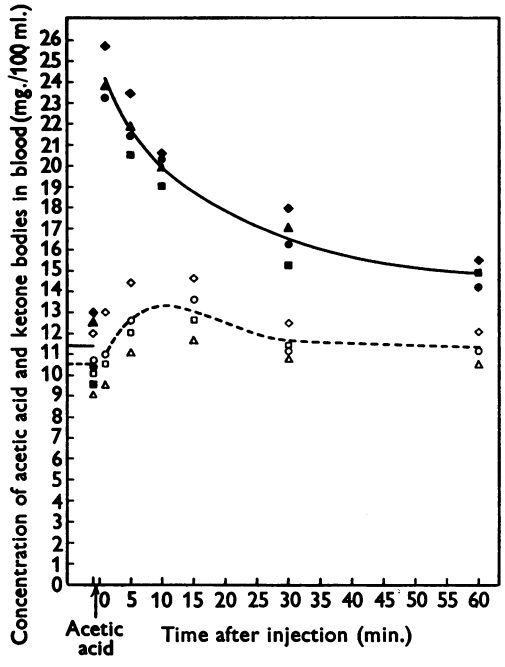

Fig. 3. Relationship between the acetate-disappearance (——) and the formation of ketone bodies (- - - -) in depancreatized dogs. The amount of acetic acid injected was 100 mg./kg. body weight. Points represent values for single animals.

Experiments were next carried out in which the concentrations of both acetate and ketone bodies in blood were measured at suitable time intervals following an injection of acetate. Fig. 2 shows the results obtained with normal dogs and Fig. 3 with diabetic dogs, respectively. It is evident that in normal dogs the blood ketone level rises at the time corresponding to the maximal drop of blood acetate and very rapidly returns to the initial values (Fig. 2). In the diabetic dogs the concentration of ketone bodies in the blood, much higher than normal even before acetate injection, increases much more than the normal and tends to return to the initial values more slowly (Fig. 3).

No significant change following the injection of acetate has ever been observed in the concentrations of glucose, phosphate and cholesterol in the blood of both the normal and depancreatized dogs.

DISCUSSION

The data show that acetate, injected into the blood stream, disappears very rapidly and that the amount of the injected acetate eliminated by the urine is very small. Although it is possible that a part of the injected acetate is secreted with the

various digestive juices, such a possibility does not explain the rapid disappearance of acetate from the blood, since the acetate is rapidly absorbed from the intestine, especially by the lymphatic system (Fonnesu & Ciaranfi, 1952; Fonnesu, 1953*a*). It may be concluded that the injected acetate is rapidly metabolized by the tissues. This conclusion is in agreement with the results of analogous experiments with labelled acetate, in which the acetate utilization was determined by the rate of incorporation of ^{14}C in those compounds of which acetate is the precursor. The concentration of acetate in the blood of diabetic dogs is greater than that of normal dogs, and acetate is utilized more slowly in the former than in the latter. The acetic acid concentration in the urine is also increased in the diabetic dog, this being in agreement with previous observations on the urine of human diabetics (Caselli & Ciaranfi, 1946).

These results may be interpreted in a number of ways, viz. in the diabetic animal (*a*) the level of CoA is lowered, (*b*) the acetylation of CoA is reduced, (*c*) the utilization of Ac~CoA is impaired, or (*d*) more than one of the above-mentioned mechanisms are involved. These hypotheses can be discussed in the light of previous data found in the literature. A reduced synthesis of CoA in diabetes, although not demonstrated, appears probable if it is assumed that the synthesis of this substance requires high-energy phosphate bonds, the production of which is lowered in diabetes because of impaired glucose oxidation (Kaplan & Greenberg, 1944; Kaplan, Franks & Friedgood, 1945; Cutolo & Siliprandi, 1952). The reduced synthesis of CoA could also be due to a diminished availability of its precursors, and it is known that in diabetes the amounts of SH compounds are reduced (Lazarow, 1949). On the other hand, the acetylation of CoA could be diminished in diabetes since the formation of Ac~CoA requires adenosine triphosphate (ATP) (Lipmann, 1945). Charalampous & Hegsted (1949*a*, *b*) have shown that in the alloxan-diabetic rat the acetylation of *p*-aminobenzoic acid is markedly reduced and that acetylation is restored to normal values by the injection of ATP, acetylphosphate, and some di- and tri-carboxylic acids of Krebs's cycle, the most active of which is malate. Insulin also reactivates, although more slowly, the acetylation processes in diabetes. None of these substances influences the rate of acetylation in the normal rat. These observations support the hypothesis that in diabetes the amount of Ac~CoA is reduced because of the reduction of oxidative phosphorylation necessary for the synthesis of CoA and for its acetylation with acetate.

The stages after Ac~CoA have been more extensively studied in diabetes. It is well known that the synthesis of higher fatty acids is markedly reduced (Brady & Gurin, 1950), that the coupling with oxalacetate must be decreased because of oxalacetate deficiency (Frohman, Orten & Smith, 1951) and that the acetylation processes are greatly diminished (Charalampous & Hegsted, 1949*a*, *b*). On the other hand, the cholesterol synthesis, which needs CoA (Klein & Lipmann, 1953), is not lowered in diabetes as compared with the normal (Brady & Gurin, 1950), whilst the level of ketone bodies, the synthesis of which requires CoA (Stadtman, Doudoroff & Lipmann, 1951) is greater in diabetic than in normal animals. It is to be noted here that our results demonstrate that the injection of acetate leads, in the diabetic animal, to a higher and more prolonged increase in the blood ketone level as compared with the normal. It is probable, however, that the high level of ketones does not depend so much upon the increased synthesis of the just mentioned substances but upon the reduced ketolysis.

It may be concluded from the above data that the reduced acetate utilization in the diabetic dog depends on many factors, among which the most important probably is the reduced formation of Ac~CoA. Experiments to clarify this point are still in progress.

SUMMARY

1. Acetate utilization, both in normal and depancreatized dogs, has been studied *in vivo* by following the rate of disappearance of acetate injected directly into the blood stream.

2. Acetic acid concentration, both in the blood and in the urine of diabetic animals, was found to be higher than normal even before acetate injection.

3. Injected acetate always disappeared from the blood very rapidly with a rate which depended upon the initial blood concentration of the compound.

4. The amount of injected acetate eliminated by way of the kidneys was small.

5. The rate of disappearance of acetate from the blood, was markedly reduced in diabetes.

6. Acetate injection produced in diabetic animals a higher and more prolonged increase in the concentration of blood ketone bodies as compared with the normal. The highest blood concentration of ketone bodies was observed at a time corresponding to the maximal drop of blood acetate.

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REFERENCES

Bertho, A. & Grassmann, W. (1936). *Biochemisches Praktikum*, p. 71, 1st ed. Berlin and Leipzig: W. de Gruyter and Co.

Bloch, K. & Rittenberg, D. (1942). *J. biol. Chem.* **145**, 625.

Bloor, W. R. (1916). *J. biol. Chem.* **24**, 227.

Brady, R. O. & Gurin, S. (1950). *J. biol. Chem.* **187**, 589.
Bruggen, J. T. van, Claycomb, C. K., Hutchens, T. T. & West, E. S. (1952). *Fed. Proc.* **11**, 301.
Buchanan, J. M., Hastings, A. B. & Nesbett, F. B. (1943). *J. biol. Chem.* **150**, 413.
Caselli, P. & Ciaranfi, E. (1946). *Arch. Sci. biol., Bologna*, **31**, 302.
Charalampous, F. C. & Hegsted, D. M. (1949*a*). *Fed. Proc.* **8**, 379.
Charalampous, F. C. & Hegsted, D. M. (1949*b*). *J. biol. Chem.* **180**, 623.
Ciaranfi, E. & Fonnesu, A. (1952). *Biochem. J.* **50**, 698.
Coniglio, J. G., Anderson, C. E. & Robinson, C. S. (1952). *J. biol. Chem.* **198**, 525.
Cutolo, E. & Siliprandi, N. (1952). *Experientia*, **8**, 24.
Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
Folin, O. & Wu, H. (1919). *J. biol. Chem.* **38**, 106.
Fonnesu, A. (1951). *Bull. Soc. Chim. biol., Paris*, **33**, 1021.
Fonnesu, A. (1953*a*). *Arch. Sci. biol., Bologna*, **37**, 107.
Fonnesu, A. (1953*b*). *Biochem. Z.* **324**, 512.
Fonnesu, A. & Ciaranfi, E. (1952). *Arch. Sci. biol., Bologna*, **36**, 335.
Frohman, C. E., Orten, J. M. & Smith, A. H. (1951). *J. biol. Chem.* **193**, 803.
Gould, R. G., Sinex, F. M., Rosenberg, I. N., Solomon, A. K. & Hastings, A. B. (1949). *J. biol. Chem.* **177**, 295.
Haberland, H. F. O. (1934). *Die operative Technik des Tierexperimentes*, p. 390, 2nd ed. Berlin and Wien: Urban and Schwarzenberg.
Hevesy, G., Ruyssen, R. & Beeckmans, M. L. (1951). *Experientia*, **7**, 144.
Hutchens, T. T. & van Bruggen, J. T. (1952). *Fed. Proc.* **11**, 235.
Kaplan, N. O., Franks, M. & Friedgood, C. E. (1945). *Science*, **102**, 447.
Kaplan, N. O. & Greenberg, D. M. (1944). *J. biol. Chem.* **156**, 525.
Klein, H. P. & Lipmann, F. (1953). *J. biol. Chem.* **203**, 101.
Lazarow, A. (1949). *Physiol. Rev.* **29**, 48.
Lifson, N., Lorber, V., Sakami, W. & Wood, H. G. (1948). *J. biol. Chem.* **176**, 1263.
Lipmann, F. (1945). *J. biol. Chem.* **160**, 173.
Lynen, F. & Reichert, E. (1951). *Angew. Chem.* **63**, 47.
Lynen, F., Reichert, E. & Rueff, L. (1951). *Liebigs Ann.* **574**, 1.
Pearson, O. H., Hastings, A. B. & Bunting, H. (1949). *Amer. J. Physiol.* **158**, 251.
Pihl, A., Bloch, K. & Anker, H. S. (1950). *J. biol. Chem.* **183**, 441.
Rittenberg, D. & Bloch, K. (1944). *J. biol. Chem.* **154**, 311.
Rittenberg, D. & Bloch, K. (1945). *J. biol. Chem.* **160**, 417.
Stadtman, E. R., Doudoroff, M. & Lipmann, F. (1951). *J. biol. Chem.* **191**, 377.
Stern, J. R. & Ochoa, S. (1949). *J. biol. Chem.* **179**, 491.
Swendseid, M. E., Barnes, R. H., Hemingway, A. & Nier, A. O. (1942). *J. biol. Chem.* **142**, 47.
Villee, C. A. & Hastings, A. B. (1949). *J. biol. Chem.* **181**, 131.
Weichselbaum, T. E. & Somogyi, M. (1941). *J. biol. Chem.* **140**, 5.
Wick, A. N. & Drury, D. R. (1952). *J. biol. Chem.* **199**, 127.