Determinations of the carboxylase activity of the yeast indicate that no quantitative adaptation had occurred as a result of growth in the presence of cocaine.

SUMMARY

1. The effects of cocaine and of added vitamin B_1 on the glucose fermentation of *Saccharomyces cerevisiae* at pH 7, previously grown in the presence and in the absence of cocaine, have been compared. There is a difference in behaviour towards added vitamin B_1 between the normal yeast and the cocaine-grown yeast.

2. Cocaine in concentration sufficient to arrest growth (0·005M) at pH 7 inhibits completely the carboxylase system of *S. cerevisiae*.

3. The carboxylase systems of yeast grown in the presence and in the absence of cocaine are inhibited by cocaine to a similar extent.

4. No quantitative adaptation of the carboxylase system of *S. cerevisiae* has been found as a result of growth in the presence of cocaine at pH 7.

REFERENCES

Atkin, L., Schultz, A. S. & Frey, C. N. (1939). *J. biol. Chem.* **129**, 471.
Beevers, H. & Simon, E. W. (1949). *Nature, Lond.*, **163**, 408.
Cook, E. S. & McDevitt, M. (1945). *Stud. Inst. Divi. Thomae.* **4**, 107.
Cruz, J. M. (1946). *Arch. portug. Sci. biol.* **8**, suppl. 32–3.
Danielli, J. F. (1950). *Cell Physiology and Pharmacology.* London: Elsevier.
Davies, B. M. A. & Yudkin, J. (1951). *Nature, Lond.*, **167**, 117.
Dienert, F. (1900). *Ann. Inst. Pasteur*, **14**, 139.
Dubos, R. S. (1940). *Bact. Rev.* **4**, 1.
Gale, E. F. (1943). *Bact. Rev.* **7**, 139.
Green, D. E., Herbert, D. & Subrahmanyan, V. (1941). *J. biol. Chem.* **138**, 327.
Hinshelwood, C. (1950). *Nature, Lond.*, **166**, 1089.
Lipton, M. A. & Elvehjem, C. A. (1940). *J. biol. Chem.* **136**, 637.
Ochoa, S. & Peters, R. A. (1938). *Biochem. J.* **32**, 1501.
Robertson, W. B. (1942). *Science*, **96**, 93.
Rona, P. & Bloch, E. (1921). *Biochem. Z.* **118**, 185.
Rona, P. & Grassheim, K. (1923). *Biochem. Z.* **140**, 493.
Ryman, B. E. & Walsh, E. O'F. (1951). *Nature, Lond.*, **167**, 770.
Spiegelman, S. (1948). *Symp. Soc. exp. Biol.* **2**, 286.
Spiegelman, S. (1950). *The Enzymes*, Vol. I, pt. 1. New York: Academic Press.
Stoppani, A. D. M. (1951). *Nature, Lond.*, **167**, 653.
Travell, J. (1940). *J. Pharmacol.* **69**, 21.

Fat Utilization by Muscle

By E. WERTHEIMER and VICTORIA BEN-TOR
Department of Pathological Physiology, Hebrew University, Jerusalem, Israel

(*Received* 19 *June* 1951)

It has generally been stated that the primary function of the liver in the catabolism of the fatty acids is to convert them into ketone bodies. Only the further oxidation of these intermediate substances was presumed to represent an extra-hepatic function. On the other hand, it has long been questioned whether muscle can also oxidize higher fatty acids directly. Gemmill (1942) found no experimental evidence to indicate the direct utilization of fat by muscle, but this view was not shared by Stadie in his review of this subject in 1945. Cruickshank & Kosterlitz (1941) were able to demonstrate that when cardiac glycogen was low the heart was able to utilize stored fatty acids for its metabolic needs. Lehninger (1946) showed that a rat-heart muscle suspension oxidizes octanoate, laurate and palmitate. Geyer, Matthews & Stare (1949) demonstrated the oxidation of octanoate and trilaurin by various extra-hepatic tissue slices; and Grafflin & Green (1948) had previously shown that kidney enzyme preparations rapidly oxidize all of the saturated monocarboxylic fatty acids. In a recent investigation Goldmann *et al.* (1950) undertook the study of the extent to which the extra-hepatic tissues of the intact animal participate in the oxidation of physiologically occurring fatty acids. Palmitic acid labelled with ^{14}C at the carboxyl position was used, and its conversion to $^{14}CO_2$ was compared in normal and in hepatectomized dogs. Evidence was presented for the view that less than 40 % of the $^{14}CO_2$ exhaled by the normal dog could have resulted from direct oxidation of the labelled palmitic acid by all the extra-hepatic tissues of the animal. At the same time a study of the oxidation of isotopic palmitic acid by various animal tissues was published by Weinhouse, Millington & Volk (1950). Palmitic acid was found to be oxidized *in vitro* by a variety of tissues besides liver; for example, by pigeon-breast muscle, but not by rat skeletal muscle or by rat brain.

In the present study the question of the direct oxidation of fatty acids by muscle *in vitro* is approached from a different angle: we employed Gemmill's diaphragm preparation which makes use of surviving muscle, with serum lipids as substrate, i.e. the natural form of lipids in the living organism.

EXPERIMENTAL

Experimental animals. The experiments were carried out with 3- to 3·5-month-old male rats weighing 90–120 g., from a strain which attains a mature weight of about 250–300 g. in 6–7 months. The animals were maintained under identical conditions of nutrition (wheat and vegetables) and temperature, for at least 1 month before the experiment. The rats were deprived of food for 17 hr. before the experiment. For the experiment the rat was anaesthetized with 1 ml. of 1% pentobarbital, opened and bled by cutting the aorta. The blood was then centrifuged and the serum collected on ice. Another rat, which had been treated identically, was then killed and its diaphragm removed and incubated in the serum of the first rat. By this method the diaphragm was not kept outside the body before incubation any longer than necessary.

Preparation of the diaphragms was carried out according to Gemmill (1940) and Tuerkischer & Wertheimer (1948).

Preparation of the abdominal muscle. In certain cases we replaced the rat diaphragm by the thin abdominal musculature of mice weighing 13–18 g. The abdominal muscle was excised together with the skin which was then peeled off. The muscle was trimmed to give a piece of muscle about 2–3 cm. long and 0·5 cm. wide. This was then cut along the abdominal midline into two nearly equal pieces of tissue. The surrounding medium consisted of the serum of a rat starved overnight before bleeding.

Incubation medium. (1) Serum (1 ml.) was incubated with the diaphragm immediately after centrifugation.

(2) Serum without glucose. About 0·5 g. of freshly washed yeast suspension was added to serum (1 ml.) which was incubated at 37° for 10 min., and subsequently centrifuged. Sugar was removed in this manner in all cases, and then the desired amount of glucose was added. The treatment of serum with yeast had no effect other than that due to removal of glucose. This was shown by the observation that oxidation of fatty acids took place only in the absence of blood glucose and that after the initial removal of blood sugar by yeast, addition of suitable quantities of glucose again abolished fatty acid oxidation.

(3) In order to induce an abnormally high fatty acid content of the serum, we maintained rats on the following diet for 12 days before the experiment: 60% margarine, 20% casein, 14% starch, 3% dried yeast and 3% salt mixture. In addition, the sera of two nephrotic patients with high fatty acid titres were employed.

Method of incubation. The incubation period was 3 hr. The incubation vessels employed were 10 ml. Erlenmeyer flasks. The diaphragms in the medium were gassed for 0·5 min. with O_2 and were then placed in a water bath at 37°, in which the flasks were shaken at the rate of 120 excursions/min.

Chemical procedure. Fat determination was carried out according to the method of Marenzi & Cardini (1943). The lipids are extracted from the blood by Bloor's method. After hydrolysis, the fatty acids are converted into lead salts by addition of lead acetate. The lead salts are converted, by the addition of chromate, into $PbCrO_4$ and this is determined colorimetrically with diphenylcarbazide in an acid medium. In our experience the experimental error of this method was 10%. Employing this method we obtained a mean total fatty acid content of 534±14 mg./100 ml. among 60 rat sera.

Strain and dietary conditions are partially responsible for the high total fatty acid content of our rat sera. We know that the values vary also seasonally. We are working now on this problem.

Measurements of glucose utilization. We used the method described by Krahl & Cori (1947). Glucose was estimated according to Somogyi (1937).

RESULTS

Decrease in fatty acids in glucose-free medium. After incubation for 3 hr., the fatty acid content of the sera had fallen by 23% (Table 1). After 2 hr. incubation the decrease was 21%; while after 5–7 hr. (four experiments) the decrease was 20%. When the simultaneous decreases in fatty acids in the diaphragm and in the surrounding serum were compared, it was found that the fall in titre of the serum

Table 1. *Decrease of fatty acids in serum of varying glucose concentrations, incubated with the diaphragm at 37° for 3 hr.*

No. of experiments	Glucose concn. of serum (mg./100 ml.)	Decrease of serum fatty acids (mg./100 mg. diaphragm)	Decrease of fatty acids in serum (%)
46	0	1·85±0·015	23
5	25	1·70±0·03	18
5	40–50	1·50±0·17	15
14	100	0·43±0·19	6

Table 2. *Decrease of fatty acids in the diaphragm and in serum, after incubation of the diaphragm in glucose-free serum for 3 hr. at 37°*

Exp. no.	Weight of diaphragm (mg.)	Decrease of fatty acids in diaphragm (mg.)	Decrease of fatty acids in serum (mg.)	Decrease of fatty acids Total (mg.)
1	55	+0·430	−0·850	−0·420
2	65	−0·153	−1·40	−1·553
3	63	−0·135	−0·70	−0·835
4	56	−0·440	−0·80	−1·240
5	53	−0·080	−1·10	−1·180
6	81	−0·020	−0·87	−0·890
7	76	−0·180	−0·87	−1·050

was almost always accompanied by a slight decrease in the diaphragm. An absolute total decrease in fatty acids was always obtained (Table 2). When mince of diaphragm or of another muscle was employed, the fall in fatty acids after the same incubation period could not be shown clearly.

Decrease in fatty acids with varying concentrations of blood sugar. In most cases when the serum contained 100 mg. glucose/100 ml., no decrease was observed in serum fatty acids, the mean fatty acid decrease falling within the range of experimental error of the method. With a serum glucose content of 40–50 mg./100 ml. a decrease in fatty acids was demonstrable; and such a decrease was even more marked when the serum glucose content was 25 mg./100 ml. (Table 1). The utilization of sugar by the diaphragm fell with the decrease in the glucose concentration of the serum (Somogyi, 1949). Similar results were obtained when the thin abdominal musculature of the young mouse was substituted for the diaphragm under otherwise identical conditions (Table 3).

Table 3. *Decrease of fatty acids in serum incubated with abdominal muscle of mice at* 37° *for* 3 *hr.*

No. of experiments	Glucose concn. of serum (mg./100 ml.)	Decrease of serum fatty acids (mg./100 mg. muscle)	Decrease of fatty acids in serum (%)
7	0	2·0 ±0·10	22
7	100	0·70±0·15	7

Inhibition of fatty acid decrease by metabolites other than glucose. Whereas fatty acid decrease is completely inhibited in the presence of 100 mg. glucose/100 ml. serum, similar quantities of galactose or of acetate were able to inhibit only partially fatty acid utilization. Pyruvate, and in particular acetoacetate, totally inhibited fat utilization (Table 4).

Table 4. *Influence of different metabolites on the decrease of fatty acids in glucose-free serum incubated with the diaphragm for* 3 *hr. at* 37°

Metabolite added (100 mg./100 ml.)	No. of experiments	Decrease of serum fatty acids (mg./100 mg. diaphragm)	Decrease of fatty acids in serum (%)
Galactose	6	0·79±0·31	12
Fructose	4	0·90±0·33	13
Sodium acetate	8	0·95±0·10	15
Sodium acetoacetate	10	0·33±0·10	5
Sodium pyruvate	6	0·30±0·17	5
Glucose	14	0·43±0·19	6

The effect of metabolic poisons. The addition to the serum of cyanide, fluoride, monoiodoacetate, fluoroacetate or 2:4-dinitrophenol almost entirely inhibited the decrease in fatty acids (Table 5).

Fatty acid decrease following incubation of diaphragm in sera of high fatty acid content. The sera of rats previously maintained on a high fat diet (mean fatty acid content close to 900 mg./100 ml.), and sera of two nephrotic patients (mean fatty acid content of 1425 mg./100 ml.) were used. With the rise in fat content, the decrease in fatty acids after incubation with diaphragm was greatly enhanced, and the inhibitory effect of glucose decreased; so that, even when the blood sugar content was

Table 5. *Influence of several metabolic inhibitors on decrease of fatty acids in glucose-free serum incubated with the diaphragm for* 3 *hr. at* 37°

Inhibitor	Concn. of inhibitor	No. of experiments	Decrease of serum fatty acids (mg./100 mg. diaphragm)	Decrease of fatty acids in serum (%)
None	—	46	1·85±0·015	23
NaCN	1×10^{-3}–2×10^{-3} M	5	0·60±0·08	5
NaF	2×10^{-3}–$2{\cdot}5\times10^{-2}$ M	7	0·40±0·22	4·6
Monoiodoacetate	5×10^{-3} M	4	0	0
Fluoroacetate	1×10^{-2}–3×10^{-3} M	5	0·50±0·40	4
2:4-Dinitrophenol	1×10^{-4} M	4	0·38±0·17	5

Table 6. *Decrease of fatty acids in sera, of high fatty acid concentration, and various glucose concentrations, after incubation with the diaphragm for* 3 *hr. at* 37°

No. of experiments	Glucose concn. of sera (mg./100 ml.)	Initial fatty acid concn. (average) (mg./100 ml.)	Decrease of serum fatty acids (mg./100 mg. diaphragm)	Decrease of serum fatty acids (%)
3	50	900*	4·0±0·40	26
6	70		3·0±0·10	31
6	100		0·7±0·26	3
7	0	1424†	9·5±3·9	37
7	70–100		5·5±1·3	30

* Sera of rats which received a fat-rich diet.

† Sera from patients with nephrosis.

normal or super-normal (150 mg./100 ml.), a fatty acid decrease was demonstrable following the customary incubation with diaphragm (Table 6).

DISCUSSION

The absolute decrease of fatty acids in glucose-free serum incubated with rat diaphragm, together with the well-known fact that the R.Q. of the diaphragm of fasting rats when equilibrated without glucose almost invariably yields the theoretical value for fat oxidation (0·7) (Stadie, 1945), leads to the conclusion that the diaphragm is capable of attacking fatty acids directly. Since similar results are obtainable with abdominal muscle of mice, it is permissible to generalize that muscle may be capable of directly breaking down fatty acids. The arrest of fatty acid decrease in the presence of cyanide argues in favour of the assumption that serum fatty acids are oxidized by muscle. Fatty acid decrease is completely inhibited when the normal quantity of blood sugar (100 mg./100 ml.) is present in the serum. In the presence of 40–50 mg. of sugar, fatty acid utilization is demonstrable; and in the presence of 25 mg. it becomes very marked. This fact leads to the problem of the integration of fat and carbohydrate metabolism, in which competition may play an important role. This question has been discussed at some length by Edson (1935) in connexion with studies on hepatic tissue. The supposition is that carbohydrates and fatty acids are in competition for the oxygen available to the normal tissue, and that when both substrates are present carbohydrate is utilized in preference to fatty acids. This idea is not supported by the studies of Weinhouse *et al.* (1949). They compared the rates of oxidation of isotopically labelled short-chain fatty acids in liver slices from previously starved rats with the rates in liver slices of well-fed rats, and they found that oxidation of the fatty acids tended to increase rather than to decrease in the latter case.

According to the results of our study, a competition exists between glucose and fatty acid oxidation in muscle. Pyruvate is also able to inhibit fatty acid oxidation; inhibition can also be shown in the presence of acetoacetate. The possibility exists of an auto-regulation preventing the breakdown of over-large quantities of fat by muscle when a great amount of the ketone bodies is already present.

If, however, an abnormally high quantity of fatty acid is present in the serum, fatty acids are broken down even in the presence of normal or excessive amounts of blood sugar. The amount of fatty acids oxidized under these circumstances is even greater than in the case of normal fat content in the absence of glucose. A rise in fatty acids in the blood may thus be prevented by means of increased breakdown by muscle. Any protracted increase of fatty acids in the blood must therefore be associated either with some disturbance of this breakdown or with an abnormally high mobilization of the fatty acid reserves.

SUMMARY

1. The direct breakdown of the fatty acids of rat serum by resting surviving muscle (diaphragm) has been studied.

2. Fatty acids in the serum decrease when diaphragm is incubated in glucose-free serum for 3 hr. No such decrease is observed when the glucose content of the serum is normal (100 mg./100 ml.). In the presence of 40–50 mg. glucose in 100 ml. serum the decrease is definitely demonstrable; and it is marked in the presence of 25 mg./100 ml.

3. As in the case of glucose, pyruvic acid and particularly acetoacetate completely inhibit fatty acid decrease; whereas inhibition by galactose, fructose and acetate is only partial.

4. Certain metabolic poisons in the customary concentrations completely inhibit the decrease of fatty acids.

5. When diaphragm is incubated in serum of abnormally high fatty acid content and with varying contents of glucose, fatty acid decrease becomes more marked and is less dependent on the glucose content of the serum, so that even with normal glucose content a fall in fatty acids is demonstrable.

REFERENCES

Cruickshank, E. W. H. & Kosterlitz, H. W. (1941). *J. Physiol.* **99**, 208.
Edson, N. L. (1935). *Biochem. J.* **29**, 2082.
Gemmill, C. L. (1940). *Johns Hopk. Hosp. Bull.* **66**, 232, 329.
Gemmill, C. L. (1942). *Physiol. Rev.* **22**, 32.
Geyer, R. P., Matthews, L. W. & Stare, F. I. (1949). *J. biol. Chem.* **180**, 1037.
Goldman, D. S., Chaikoff, J. L., Reinhardt, W. O., Entenman, C. & Daubeń, W. G. (1950). *J. biol. Chem.* **184**, 719.
Grafflin, A. L. & Green, D. E. (1948). *J. biol. Chem.* **176**, 95.
Krahl, M. E. & Cori, C. F. (1947). *J. biol. Chem.* **170**, 607.
Lehninger, A. L. (1946). *J. biol. Chem.* **165**, 131.
Marenzi, A. D. & Cardini, C. E. (1943). *Rev. soc. argent. Biol.* **19**, 118.
Somogyi, M. (1937). *J. biol. Chem.* **117**, 771.
Somogyi, M. (1949). *J. biol. Chem* **174**, 597; **179**, 217.
Stadie, W. C. (1945). *Physiol. Rev.* **25**, 395.
Tuerkischer, E. & Wertheimer, E. (1948). *Biochem. J.* **42**, 603.
Weinhouse, S., Millington, R. H. & Friedman, B. (1949). *J. biol. Chem.* **181**, 489.
Weinhouse, S., Millington, R. H. & Volk, M. E. (1950). *J. biol. Chem.* **185**, 191.