The Effect of Ketone Bodies on the Carbohydrate Metabolism of Rat Diaphragm

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High blood levels of ketone bodies have been associated with 'toxicity' in diabetic acidosis and other acidotic states. This toxicity has never been adequately explained, particularly in chemical terms, and there have been only few experimental investigations of the problem. Nath & Brahama-chari (1944) and Nath & Chakrabarti (1950) injected small doses of acetoacetate and β -hydroxybutyrate into rabbits over a prolonged period. A depletion of stored glycogen was found in the injected animals as compared with controls. Tidwell & Axelrod (1948) administered small quantities of acetoacetate to rats for a shorter time and observed no effect.

In a preliminary note (Chari & Wertheimer, 1953), we reported the effects of ketone bodies on carbohydrate metabolism, in particular at concentrations comparable with those found in diabetic acidosis. This condition was obtained in the *in vivo* experiments by a single large injection of one of the ketone bodies. For the *in vitro* experiments, an equivalent amount of the respective ketone body was added to the incubation medium.

The purpose of the work recorded here is to determine whether ketone bodies are merely end-products of abnormal metabolism or metabolites which during their accumulation influence normal metabolism (Parnes & Wertheimer, 1950).

EXPERIMENTAL

Animals

Albino male rats of an inbred strain, weighing 80–120 g. when 3–3.5 months old, were used. Food was withheld for 20 hr. before the experiment. The rats were anaesthetized with pentobarbitone and bled.

In vitro experiments

Incubation of the diaphragm was by the method described by Gemmill (1940). The medium consisted of 1 ml. phosphate-buffered saline containing 0.74% NaCl, 0.015% Na_2HPO_4 and 0.003% NaH_2PO_4 , pH 7.4 (Tuerkischer & Wertheimer, 1948). The diaphragms and medium were put into 10 ml. Erlenmeyer flasks and gassed for 30 sec. with oxygen. The flasks were then placed in a water bath at 37° and shaken at the rate of 120 excursions/min. for approximately 2.5 hr.

Substrates. All substrates were used as sodium salts. Sodium acetoacetate was prepared according to Ljunggren (1924), and calculated as acetoacetic acid. Sodium β -hydroxybutyrate was a product of British Drug Houses Ltd. The concentrations of the substrates, given in the text as % (w/v), were equivalent to about 0.008 M.

Measurement of glycogen synthesis. The measurements were carried out according to Tuerkischer & Wertheimer (1948).

Measurement of glycogenolysis. These experiments were performed in the phosphate-buffered solution mentioned above, without adding glucose. When serum was used as the medium, it was freed of glucose by incubation with yeast for 10 min. at 37°.

Measurement of glucose utilization. The method described by Krahl & Cori (1947) and Perlmuter & Greep (1948) was used. Values of glycogen synthesis, glycogenolysis and glucose utilization are all expressed in mg. glucose/100 mg. wet tissue.

Measurement of fatty acid oxidation. Serum was chosen as a medium for the experiments on fatty acid oxidation, following the method of Wertheimer & Bendor (1952). Glucose was removed by fermentation with yeast and the ketone bodies were added. Serum without ketone bodies was employed as control. The diaphragm was incubated in this serum for 2.5 hr. at 37°. Fatty acid oxidation was expressed as mg. fatty acid/100 mg. wet tissue.

Respiration measurements. Determination of oxygen uptake was carried out by Warburg's direct method (Dixon, 1951), in phosphate-saline medium. Measurements were made over periods varying from 2 to 3 hr., and the average value for 1 hr. was calculated.

Measurement of glycogen synthesis in liver slices. Liver slices, prepared with a tissue slicer (Stadie & Riggs, 1944), were incubated for 3 hr. in 1 ml. of the medium described by Buchanan, Hastings & Nesbett (1949) or normal rat serum. The concentration of glucose was 0.5% and of acetoacetate, 0.1% or 0.5%. The flasks were gassed with 95% O_2 + 5% CO_2 and placed in a water bath at 37°.

In vivo experiments

In order to raise the level of ketone bodies *in vivo* several methods were employed:

(a) Ketone bodies in saline were administered intraperitoneally to rats fasted for 20 hr. The dose was 100 mg./100 g. body wt., since lesser quantities were without obvious effect. Control animals received saline and acetate.

(b) Diabetes was induced by intravenous injection of 5.5 mg. alloxan/100 g. body wt. Animals were used in various stages of diabetes ranging from severe coma to latent diabetes. They were fasted for 20 hr. before use.

(c) Rats were fasted for 1-7 days. The rats fasted for 7 days lost about 36% of their weight and were in poor condition.

(d) Rats were fed for 6-12 days on the following diet: 62% fat (margarine), 21% casein, 10% carbohydrate (starch), 4% salt mixture and 3% dried yeast. After the rats had been fasted for 20 hr. the experiments were carried out in the usual way.

Chemical determinations

Glycogen in the diaphragm was estimated by the method of Good, Kramer & Somogyi (1933); glucose, according to Somogyi (1937); total fatty acids, according to Stern & Shapiro (1953); ketone bodies, by the method of Engfeld, described by Rappaport (1949). The later values were given in mg. acetone/100 ml.

RESULTS

Effect of ketone bodies on the glycogen content of rat diaphragm with and without glucose

Addition of 0.1% acetoacetate to phosphate-buffered medium containing 0.1% glucose was accompanied by a decrease in the extent of glycogen synthesis. Addition of 0.05% acetone or 0.1% β -hydroxybutyrate had no effect on glycogen synthesis, while 0.25% β -hydroxybutyrate was stimulatory.

In the absence of glucose 0.1% acetoacetate had

no effect on the rate of glycogenolysis. Under similar conditions, 0.1% β -hydroxybutyrate or 0.05% acetone markedly inhibited the rate of glycogenolysis (Fig. 1).

A similar effect was observed with liver slices. When incubated in normal rat serum or in the medium of Buchanan *et al.* (1949) containing 0.5% glucose, 0.1% acetoacetate did not significantly affect glycogen synthesis (0.199 ± 0.014 with acetoacetate, 7 expts.; 0.213 ± 0.013 without acetoacetate, 10 expts.). Addition of 0.5% acetoacetate, however, significantly decreased glycogen synthesis (0.173 ± 0.009 , 8 expts.) in the medium of Buchanan *et al.* (1949).

This decrease of glycogen synthesis in the presence of acetoacetate will henceforth be called the 'acetoacetate effect'.

Effect of varying glucose and acetoacetate concentrations on glycogen synthesis by rat diaphragm

The 'acetoacetate effect' was found to be dependent on the relative concentrations of glucose and acetoacetate in the medium. The effect was diminished by (a) increasing the concentration of glucose relative to that of acetoacetate, or (b) decreasing the concentration of acetoacetate relative to that of glucose (Table 1).

Fig. 1. Effect of ketone bodies on the glycogen content of rat diaphragm in presence and absence of glucose. ---, glycogen content in the presence of 0.1% glucose but no other substrate; - - -, glycogen content without addition of any substrate. Φ , Glycogen content when one substrate—but not glucose—is added. $\bar{I}$, Glycogen content when a substrate and glucose are added.

Table 1. *Effect of varying glucose and acetoacetate concentrations on glycogen synthesis of rat diaphragm*

The diaphragms were incubated at 37° for 2.5 hr. Glycogen synthesis is expressed as mg. glucose/100 mg. wet tissue $\pm$ standard error of the mean.

Varying glucose concentration				Varying acetoacetate concentration			
No. of measurements	Glucose (g./100 ml.)	Aceto-acetate (g./100 ml.)	Glycogen synthesis	No. of measurements	Glucose (g./100 ml.)	Aceto-acetate (g./100 ml.)	Glycogen synthesis
7	0.05	0	0.098 $\pm$ 0.012	—	—	—	—
7	0.05	0.10	0.007 $\pm$ 0.008	—	—	—	—
12	0.10	0	0.136 $\pm$ 0.011	12	0.10	0	0.136 $\pm$ 0.011
12	0.10	0.10	0.042 $\pm$ 0.007	5	0.10	0.025	0.125 $\pm$ 0.038
7	0.20	0	0.184 $\pm$ 0.029	7	0.10	0.05	0.076 $\pm$ 0.010
12	0.20	0.10	0.175 $\pm$ 0.014	12	0.10	0.10	0.042 $\pm$ 0.007

Table 2. *Effect of ketone bodies on glucose utilization of rat diaphragm*

The diaphragms were incubated at 37° for 2.5 hr. Final concentrations: glucose, acetoacetate and β -hydroxybutyrate, 0.1 g./100 ml.; acetone, 0.05 g./100 ml.

No. of measurements	Medium	Glucose utilization (mg./100 mg. wet tissue)
30	Glucose	0.50 $\pm$ 0.003
11	Glucose + β -hydroxybutyrate	0.54 $\pm$ 0.009
6	Glucose + acetone	0.52 $\pm$ 0.009
21	Glucose + acetoacetate	0.57 $\pm$ 0.004

Table 3. *Effect of ketone bodies on the oxygen uptake of rat diaphragm*

The diaphragms were incubated in phosphate-saline containing either 0.1 % acetoacetate or 0.1 % β -hydroxybutyrate with or without the addition of 0.1 % glucose; control contained either 0.1 % glucose alone or nil.

No. of measurements	Medium	Q_{O_2} (μ l./mg. dry wt./hr.)
3	Nil	-6.31 $\pm$ 0.23
9	Glucose	-6.12 $\pm$ 0.10
6	Acetoacetate	-7.33 $\pm$ 0.10
6	Acetoacetate + glucose	-7.84 $\pm$ 0.12
5	β -Hydroxybutyrate	-6.44 $\pm$ 0.60
5	β -Hydroxybutyrate + glucose	-7.00 $\pm$ 0.43

Table 4. *Effect of ketone bodies on the oxidation of fatty acids*

The diaphragms were incubated at 37° for 2.5 hr. in glucose-free normal rat serum. Final concentrations of acetoacetate, and β -hydroxybutyrate 0.1 %, acetone, 0.05 %.

No. of measurements	Substrate	Fatty acid oxidation (mg./100 mg. wet tissue)
8	Nil	0.73 $\pm$ 0.007
7	Acetoacetate	0.60 $\pm$ 0.009
7	β -Hydroxybutyrate	0.31 $\pm$ 0.006
4	Acetone	0.28 $\pm$ 0.027

Effect of ketone bodies on the glucose utilization and oxygen uptake of rat diaphragm

Addition of 0.1 % acetoacetate caused a slight but significant increase in glucose utilization; 0.1 % β -hydroxybutyrate or 0.05 % acetone showed no such effect (Table 2).

The oxygen uptake was greater in the presence of 0.1 % acetoacetate than in the presence of 0.1 % glucose, and was greater still in the presence of both acetoacetate and glucose. β -Hydroxybutyrate behaved similarly to acetoacetate, but the effect was not so marked (Table 3).

Effect of ketone bodies on the oxidation of fatty acids by rat diaphragm

Oxidation of fatty acids was measured in normal rat serum after removal of glucose. Compared with the control value, acetoacetate caused only a slight decrease in the rate of oxidation, while β -hydroxybutyrate and acetone caused about 50 % inhibition (Table 4).

Influence of ketone bodies on glycogen synthesis and glucose utilization of rat diaphragm in the presence of insulin and adrenalin

Insulin (0.1 unit) increased glycogen synthesis by the rat diaphragm in the presence of glucose, as first reported by Gemmill (1940). Acetoacetate (0.1 %) diminished this stimulation, but was without effect on glucose utilization (Fig. 2). β -Hydroxybutyrate and acetone did not affect the stimulation of glycogen synthesis by insulin.

This effect of adrenalin on glycogen synthesis by rat diaphragm was not influenced by the addition of acetoacetate. When 0.1 mg./100 ml. adrenalin and 0.1 % glucose were present, a value of -0.003 ± 0.033 was obtained in six expts.; addition of 0.1 % acetoacetate gave a value of 0.015 ± 0.025 in 8 expts.

Fig. 2. Effect of acetoacetate on glycogen synthesis and glucose utilization in the presence of insulin.

Table 5. *Effect of β -hydroxybutyrate on the 'acetoacetate effect'*

Diaphragms were incubated at 37° for 2.5 hr. Glycogen synthesis is expressed as mg. glucose/100 mg. wet tissue. Glucose, acetoacetate and β -hydroxybutyrate all used at a final concn. of 0.1 %.

No. of measurements	Additions	Glycogen synthesis
6	Acetoacetate	0.053 ± 0.013
10	β -Hydroxybutyrate	0.130 ± 0.016
8	Acetoacetate + β -hydroxybutyrate	0.144 ± 0.018

Effect of β -hydroxybutyrate on the 'acetoacetate effect' in diaphragms of normal and diabetic rats

β -Hydroxybutyrate (0.1 %) in a medium containing 0.1 % acetoacetate and 0.1 % glucose diminished the 'acetoacetate effect' (Table 5). β -Hydroxybutyrate (0.1 %) in media containing diaphragms of diabetic rats caused an increase of glycogen synthesis in the presence of 0.1 % glucose (0.149 ± 0.019 in 7 expts. with β -hydroxybutyrate; 0.104 ± 0.015 in 7 expts. without β -hydroxybutyrate). The acetone concentration in the blood was 19 ± 3 mg./100 ml.

A similar effect of β -hydroxybutyrate had already been observed after 0.2 % was added to normal diaphragms.

Effect of methylene blue on glycogen synthesis and glucose utilization in presence and absence of acetoacetate

Methylene blue (0.0001M) decreased glycogen synthesis and increased glucose utilization as acetoacetate had done. The effects of acetoacetate and methylene blue were not additive (Table 6).

'Acetoacetate effect' in rats in different stages of diabetes

The animals were divided into two groups: (1) blood-sugar levels of 200–500 mg./100 ml., (2) blood-sugar levels of 500–1000 mg./100 ml. The 'acetoacetate effect' was clearly observed in the diaphragms of group (1) (0.065 ± 0.018 in the presence of acetoacetate, 8 expts., compared with 0.114 ± 0.026 in the absence of acetoacetate, 8 expts.). The acetone concentration in the blood was 13 ± 2 mg./100 ml. The 'acetoacetate effect' was absent from the diaphragms of group (2) (0.099 ± 0.028 with acetoacetate, 6 expts., compared with

Table 6. *Effect of methylene blue on glycogen synthesis and glucose utilization in presence and absence of acetoacetate*

The diaphragms were incubated at 37° for 2.5 hr. Glycogen synthesis and glucose utilization expressed as mg. glucose/100 mg. wet tissue. Glucose (0.1 %) present in all flasks. Additions: acetoacetate (0.1 %) or methylene blue (0.0001 M).

No. of measurements	Additions	Glycogen synthesis	Glucose utilization
10	Methylene blue	-0.074 ± 0.013	0.56 ± 0.005
6	Methylene blue + acetoacetate	-0.046 ± 0.010	0.55 ± 0.006
6	Nil	0.140 ± 0.010	0.48 ± 0.004
8	Acetoacetate	0.055 ± 0.024	0.55 ± 0.005

Table 7. *Effects of injected acetoacetate, β-hydroxybutyrate, acetate and saline of rat diaphragm on carbohydrate metabolism*

Diaphragms were incubated at 37° for 2.5 hr. Glycogen synthesis and glucose utilization are expressed as mg. glucose/100 mg. wet tissue. Acetone bodies are expressed as mg. acetone/100 ml. Glucose level is given as mg./100 ml.

No. of measurements	Substance injected	Time after injection (hr.)	Blood acetone concn.	Blood glucose concn.	Glucose utilization	Glycogen synthesis
14	Saline	0-2	7.4 ± 1	82 ± 7	0.47 ± 0.003	0.159 ± 0.014
13	Acetoacetate	0-2	27.1 ± 1	62 ± 5	0.43 ± 0.007	0.105 ± 0.015
5	β-Hydroxybutyrate	0-2	18.0 ± 2	76 ± 5	0.45 ± 0.003	0.120 ± 0.013
3	Acetate	0-2	8.0 ± 2	89 ± 5	0.44 ± 0.004	0.155 ± 0.015
5	Saline	6	6.3 ± 1	84 ± 7	0.45 ± 0.002	0.155 ± 0.028
5	Acetoacetate	6	11.0 ± 2	80 ± 6	0.53 ± 0.007	0.120 ± 0.023
8	Saline*	18-20	12.1 ± 1	70 ± 6	0.40 ± 0.005	0.070 ± 0.019
9	Acetoacetate*	18-20	13.7 ± 1	76 ± 5	0.49 ± 0.004	0.084 ± 0.012

* These experiments were carried out after 36 hr. fasting.

0.065 ± 0.012 without acetoacetate, 6 expts.). The acetone concentration in the blood was 23 ± 3 mg./100 ml.

'Acetoacetate effect' in starved rats

Rats starved 5-7 days lost about 32-38 % of their initial weight. The 'acetoacetate effect' was observed in rats fasted for 1-3 days but was not seen in those starved for 5-7 days (0.085 ± 0.021 with acetoacetate, 6 expts., compared with 0.097 ± 0.017 without acetoacetate, 6 expts.). The acetone concentration in the blood was 11 ± 3 mg./100 ml.

'Acetoacetate effect' in rats fed a high-fat diet

After 12 days on the high-fat diet, the animals were killed and their diaphragms found to exhibit a normal 'acetoacetate effect' (0.060 ± 0.017 with acetoacetate in 5 expts. compared with 0.139 ± 0.023 without acetoacetate in 5 expts.). The acetone concentration in the blood was 9.3 mg./100 ml. as compared with 5.8 mg./100 ml. after 1 day fasting.

Influence of injected acetoacetate on glycogen synthesis and glucose utilization by rat diaphragm in vitro; comparison with other injected substances

A single injection of acetoacetate (100 mg./100 g. body weight) was followed by a rise in blood acetone bodies. *In vitro* the diaphragms of these rats

showed a decreased glycogen synthesis compared with those which had been injected with equivalent amounts of acetate or with saline. In general, the higher the level of blood acetone *in vivo*, the greater the depression of glycogen synthesis *in vitro* (Table 7). This effect was still noticeable up to 6 hr. after injection, at which time the concentration of acetone bodies was approaching normal levels. The effect after 6 hr. was not significant.

The injection of equivalent doses of β-hydroxybutyrate also caused a decrease in glycogen synthesis by the rat diaphragm, but the effect was of doubtful significance ($P = 0.05$).

In Table 7 it is seen that a single injection of acetoacetate of 100 mg./100 g. body weight, caused a decrease in the blood-glucose level, thus confirming the observation of Tidwell & Axelrod (1948). On the other hand, insignificant effects were produced by the other substrates administered. These injections were followed by an alkalosis which disappeared after 3 hr.

In another series of experiments an injection of acetoacetate was followed by one of β-hydroxybutyrate. The latter cancelled the 'acetoacetate effect' (0.015 ± 0.015, 13 expts., when acetoacetate alone was administered; 0.120 ± 0.013, 5 expts., when β-hydroxybutyrate was administered; and 0.133 ± 0.028, 4 expts., when acetoacetate was followed by one of β-hydroxybutyrate).

It should be noted that a single injection of 100 mg. acetoacetate frequently caused convulsions and restlessness, as reported by Nath & Sahu (1952). These effects were seldom observed after an injection of β -hydroxybutyrate.

DISCUSSION

The present investigation shows that acetoacetate is the only ketone body which has a significant effect on glycogen synthesis in the diaphragm and liver slices of the rat, at concentrations comparable with those found in diabetic coma. This effect is noticed only within a small range of glucose concentrations relative to the concentration of acetoacetate. Similar interdependence has been found for the action of acetate on glycogen synthesis by rat diaphragm (Parnes & Wertheimer, 1950). The absence of an 'acetoacetate effect' at other relative concentrations of glucose is in agreement with the experiments of Nagler (1952) and of Tidwell, Nagler & Dunkelberg (1953).

Acetoacetate, but not β -hydroxybutyrate or acetone, causes in the rat diaphragm a slight increase of glucose utilization and oxygen consumption.

Inhibition of insulin action has been reported by Guest, Mackler & Knowles (1952) in acidotic dogs and by Duncan (1952) in acidotic diabetic patients. In our *in vitro* experiments, carried out in the presence of acetoacetate, an inhibition of insulin action has also been found. The absolute increment of glycogen synthesis due to insulin is decreased by acetoacetate (Fig. 2). However, when taking into account the initial lower glycogen synthesis, caused by acetoacetate in the absence of insulin, the relative augmentative action of insulin is not affected by acetoacetate.

In vivo experiments confirm the *in vitro* results. A single injection of acetoacetate (100 mg./100 g. body weight) causes an increased concentration of ketone bodies in the blood and a depression of glycogen synthesis by the rat diaphragm *in vitro*. This effect has been present up to 6 hr. after acetoacetate administration, at which time the concentration of blood ketone bodies has been approaching normal levels.

It is possible that acetoacetate plays the role of a hydrogen acceptor and increases glucose catabolism, concomitantly depressing glycogen synthesis. In agreement with this suggestion, β -hydroxybutyrate injected after acetoacetate or added *in vitro*, inhibits the 'acetoacetate effect' on glycogen synthesis.

In conclusion, it may be stated that acetoacetate is the only toxic ketone body. Under normal, non-pathological conditions the appearance of β -hydroxybutyrate and acetone as products of aceto-

acetate metabolism neutralizes the toxic 'acetoacetate effect'. In cases of diabetic coma or prolonged starvation, acetoacetate is produced in larger quantities than normal. Since diabetic animals utilize ketone bodies as rapidly as normal animals (Barnes, Drury, Greeley & Wick, 1940), a surplus of ketone bodies accumulates, acetoacetate being the first to appear. This surplus of acetoacetate decreases glycogen synthesis to a certain extent. Further addition of acetoacetate *in vitro* to the diaphragm of an animal suffering from diabetic coma or prolonged starvation will not increase the effect.

On the basis of our experiments it is probable that acetoacetate at high concentrations is one of the factors causing derangement of carbohydrate metabolism.

SUMMARY

1. The effect of ketone bodies on the *in vitro* carbohydrate metabolism of rat diaphragm has been investigated.

2. Acetoacetate depressed glycogen synthesis in rat diaphragm and slightly increased glucose utilization. These effects were dependent on the relative concentrations of acetoacetate and glucose.

3. Acetoacetate depressed glycogen synthesis in rat-liver slices. As with the rat diaphragm the effect was dependent on the relative concentrations of acetoacetate and glucose.

4. β -Hydroxybutyrate at a concentration equivalent to that of acetoacetate was without effect on the glycogen synthesis and the glucose utilization of rat diaphragm. Higher concentrations resulted in an increased glycogen synthesis. Addition of β -hydroxybutyrate to a medium containing acetoacetate cancelled the 'acetoacetate effect'.

5. Methylene blue, like acetoacetate, caused a decrease in glycogen synthesis and an increase in glucose utilization.

6. No 'acetoacetate effect' was found with diaphragms of severely diabetic rats, or of rats fasted for 5-7 days. Addition of β -hydroxybutyrate caused an increase of glycogen synthesis by the diaphragms of diabetic rats.

7. The oxidation of serum fatty acids by rat diaphragm was markedly depressed by β -hydroxybutyrate and acetone but only slightly by acetoacetate.

8. The stimulation by insulin of glycogen synthesis from glucose by rat diaphragm was diminished by the addition of acetoacetate.

9. Administration of acetoacetate *in vivo* depressed glycogen synthesis by the isolated rat diaphragm. The influence of acetoacetate persisted up to 6 hr. after injection. Administration of β -hydroxybutyrate *in vivo* following acetoacetate cancelled the 'acetoacetate effect'.

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The Component Acids of the Fats of Some Indian Fresh-water Fishes

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The component acids of fats from different parts of bhakur (*Catla buehanani*) were investigated by Pathak & Agarwal (1952). The fats obtained from the body and viscera of two other species of Indian fresh-water fishes have now been studied.

EXPERIMENTAL

The fresh-water fishes used were rohu (*Labeo rohita*) and nain (*Cirrhina mrigala*) caught in the River Ganges near Banaras. The body and viscera (excluding the liver) were separately cut into small pieces and exhaustively extracted with hot acetone. The extracted material was taken up in light petroleum (b.p. 40–60°), the aqueous fraction was separated and the lipids were recovered and dried at 100° under reduced pressure. The viscera of these fish contained nearly 45% of fat. The fats were kept in 10 vol. of acetone at 0° for 4 weeks to separate the phosphatides, if present, from the glycerides.

The fats were hydrolysed, and the mixed fatty acids resolved into groups of simpler mixtures of acids by the Li salt-acetone method (Tsujimoto, 1920; Tsujimoto & Kimura, 1923), followed by further resolution of the insoluble fraction by the Pb salt-ethanol method as modified by Hilditch (1949). Each group of acids was separately converted into methyl esters, with the precautions recommended by Bjarnson & Meara (1944), and fractionated at approx. 0.1 mm. pressure through an electrically heated, packed column (Longenecker, 1937). The compositions of the ester fractions were calculated from the iodine values and the saponification equivalents by the method of

Hilditch (1949). The mean unsaturation, expressed by the fractional number of hydrogen atoms short of saturation (e.g. -2.0, mono-ethenoid), was determined as usual by interpolation or extrapolation from the respective ester fractions in each group. The compositions of the original fats were obtained from those of the separated groups of fatty acids.

RESULTS

The results of the preliminary fractionation of 180 g. mixed body fatty acids and 175 g. mixed visceral fatty acids of rohu are given in Table 1. This table also gives results for 162.9 g. mixed body fatty acids and 134.7 g. mixed visceral fatty acids of nain.

The component acids of each group and of the whole of the body and the visceral fats are recorded in Tables 2 and 3 for rohu and in Tables 4 and 5 for nain.

DISCUSSION

Rohu body and visceral fats

These fats show similarities to typical fats of fresh-water fishes in having unsaturated C₁₈ acids as the predominant group, palmitic acid being the major saturated acid component. There are traces of unsaturated C₂₄ acids (Table 2), and this has also been observed in the body fat of bhakur, another species of Indian fresh-water fish (Table 6).