The Respiration of Rat-liver Slices in the Presence of Some Aliphatic Amines, Hydroxy Amines and Quaternary Ammonium Salts

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Eperjessy & Zathureczky (1947) have examined the action of certain aliphatic amines and related compounds on the aerobic metabolism of liver tissue from fasted rats. They state that these substances, when added to a medium containing liver slices respiring in oxygen, bring about a marked reduction in the value of the R.Q. without altering the level of oxygen uptake. This effect was claimed to occur at very low concentrations of added substance, a substantial reduction of R.Q. usually being found at $2 \times 10^{-5} \text{ M}$ and in some instances an effect was seen at $2.5 \times 10^{-6} \text{ M}$. The compounds tested were choline chloride, tetramethylammonium iodide and the hydrochlorides of ethylamine, trimethylamine, ethanolamine and triethanolamine, the last mentioned being the most active of the series. The authors have interpreted their results as a simultaneous stimulation of fat metabolism and inhibition of carbohydrate metabolism by the amines and other substances used.

A careful examination of the experimental data presented by Eperjessy & Zathureczky (1947) has revealed that, in certain respects, their findings are quantitatively at variance with those of other workers in this field. Attention is especially directed to the following points relating to their observations on liver slices from fasted rats, respiring in the absence of added amines, i.e. the endogenous respiration. (1) The oxygen consumption rates are very low. Although they do not use the conventional Q_{O_2} units ($\mu\text{l./mg. dry wt./hr.}$), their figures for oxygen uptake, when converted to this system, yield values in the range 3.1–3.9; this is much below the generally accepted level of about 7–10 (Edson, 1936; Bach & Holmes, 1937; Craig, 1943; Fuhrman & Crismon, 1944). (2) The R.Q. values, given as lying in the range 0.92–1.01 (four experiments), are higher than any previously reported for liver slices from fasted rats; other workers have invariably obtained much lower figures, falling within the range 0.34–0.68 (Dickens & Simer, 1931; Elliott & Baker, 1935; Elliott, Greig & Benoy, 1937).

In view of these discrepancies and the clearly important bearing of the effect obtained with choline chloride on the general problem of lipotropic action, it was felt necessary to repeat the experiments of the

Hungarian workers. Values for Q_{O_2} and R.Q. have been obtained for liver slices from fasted rats which are in complete agreement with the earlier work referred to above, and in contrast to the findings of Eperjessy & Zathureczky (1947). Seven aliphatic amines, hydroxy amines or quaternary ammonium salts, including five of those previously mentioned, have been tested at $2 \times 10^{-5} \text{ M}$ and $2 \times 10^{-2} \text{ M}$ concentration; in general, these additions did not affect the values of Q_{O_2} or R.Q. Experiments were also conducted on liver slices from well-fed rats, but again the substances tested were without effect on the respiration. Finally, the extent of carbohydrate utilization or formation (glyconeogenesis) in rat liver was determined directly, but the figures obtained were not markedly influenced by the addition of the aliphatic amines. In short, the data presented in this paper offer no support for the claims of Eperjessy & Zathureczky.

EXPERIMENTAL

Materials

Amines, hydroxy amines and quaternary ammonium salts. The best available commercial samples of dimethylamine hydrochloride, trimethylamine hydrochloride, choline chloride and tetramethylammonium iodide were employed. Specimens of ethanolamine and triethylamine were neutralized with HCl before use. Triethanolamine hydrochloride was prepared from a technical sample of the free base by treating a 20% (v/v) ethanolic solution with a slight excess of conc. HCl; the precipitated salt was twice recrystallized from aqueous ethanol.

Hexosediphosphate (HDP). A solution of the Na salt was prepared by adding the calculated amount of aqueous Na oxalate to a solution of 200 mg. of Ca HDP, dissolved in the minimum quantity of dil. HCl; the mixture was neutralized with NaOH, made up to 10 ml. with distilled water and the Ca oxalate ppt. removed by centrifugation. An earlier determination had shown 15.8% Ca in the sample of Ca HDP (calc. for $\text{C}_6\text{H}_{10}\text{O}_{12}\text{P}_2\text{Ca}_2$; Ca, 19.2%). Where this solution was used, 0.1 ml. was added to 0.9 ml. respiration medium to give a final concentration of 0.004 M.

Respiration medium. A phosphate-buffered medium was used, prepared by mixing 1 vol. of 0.2 M-Sørensen phosphate buffer, pH 7.4, with 3 vol. of 0.12 M-KCl. Thus the final medium contained 0.05 M-phosphate and 0.09 M-chloride; the atomic Na/K ratio was 0.45/0.55, and the medium was practically isotonic with mammalian tissues.

Methods

General technique. Young adult male or female rats, belonging to the hooded Rowett Institute strain, were taken from the laboratory stock; they usually weighed 200–300 g. and were maintained on an adequate diet. When fasted animals were required, food was removed from the cage 24 hr. before they were killed, but water was supplied *ad lib.*

The rats were killed by decapitation and bleeding; the two large lobes of liver were removed and transferred to ice-cold respiration medium in a small beaker surrounded by crushed ice. After 2 min. the lobes were cut into small pieces of approx. 30–40 sq.mm. cross-section. Slices were cut either by the method of Deutsch (1936), as modified by Cohen (1945), or with an instrument similar to that described by Stadie & Riggs (1944), using a 0.35 mm. microtome. (Slices cut by these methods gave similar values for Q_{O_2} and R.Q.) The slices were stored in ice-cold medium until all had been cut and were then transferred to standard Warburg flasks containing medium with or without amines or other additions; the total fluid vol. was 1.0 ml. and the dry weight of slices taken (determined after the respiration period) was about 30 mg. Q_{O_2} values were always calculated on the basis of final dry weight (on the average 23.1% of the fresh weight for both fasted and fed rats).

Conventional Warburg technique was employed, the respiration being conducted at 38° in O_2 for 1 hr. All measurements were made in duplicate and the mean taken. Average agreement between duplicates was $\pm 6.5\%$. Similar large variations between rabbit-liver slices have been noted by Crandall (1945). R.Q. values were obtained by the 'direct' method of Warburg (Dixon, 1943).

Estimation of carbohydrate. Freshly cut liver slices (100–200 mg.) were introduced into each of a series of tared 50 ml. conical flasks containing buffer, with or without added amine, of total volume 1.0 ml. The weight of tissue was found by difference. H_2SO_4 (0.33 ml., 2N) was added to each flask either initially or after the slices had been shaken 1–1.5 hr. in O_2 at 38°. In each case, the tissue and medium were transferred to a glass mortar, ground finely with a little acid-washed sand and the suspension transferred to a 10 ml. graduated centrifuge tube; the flask and mortar were repeatedly rinsed with 0.5N- H_2SO_4 and the washings added to the centrifuge tube. The fine suspension was made up to a known volume with 0.5N- H_2SO_4 and used for the determination of carbohydrate by one of the following methods.

(i) *Total carbohydrate by the anthrone method.* The procedure of Morris (1948) was modified as follows. The tissue suspension (see above) was centrifuged and a suitable volume of the very slightly opalescent supernatant fluid, containing 0.05–0.2 mg. carbohydrate, was pipetted into a large boiling tube (25×200 mm.). Distilled water was added to 5 ml. Anthrone (Meyer, 1928) (0.2% w/v in 95% v/v H_2SO_4 , 10 ml.) was then added and the contents mixed; the tube was covered with a glass bulb and immersed in a boiling water bath for exactly 10 min. After cooling, the intensity of the green-coloured solution was compared with a glucose standard and water control, similarly treated. A photoelectric colorimeter (Evans Electroelenium Ltd.) with red filter was used, the logarithmic scale reading being directly proportional to glucose concentration. The initial presence of a little protein in the test sample did not interfere, as it dissolved completely in the rather concentrated

H_2SO_4 at 100°. Morris (1948) has shown that equivalent amounts of glucose, maltose, dextrans and glycogen give identical colour intensity. This modified method of estimation was found to be very reliable.

(ii) *Total reducing substances after acid hydrolysis.* A measured volume of tissue suspension, containing 0.5N- H_2SO_4 , was placed in a boiling water bath for 3 hr. In this way, all the carbohydrate was hydrolysed to glucose (Cori & Cori, 1933). After cooling, the solution was made almost neutral by addition of 10% (w/v) NaOH and deproteinized by addition of 0.1 ml. of 30% (w/v) $HgSO_4$ in 10% (w/v) H_2SO_4 , followed by $BaCO_3$ -Zn dust treatment (West, Scharles & Peterson, 1929). A sample of the filtrate was used for the determination of total-reducing substances by the copper-iodometric method of Somogyi (1937).

Estimation of choline. Trichloroacetic acid filtrates were prepared from slices and medium. After neutralizing, the reineckate method, described by Glick (1944), was used.

RESULTS

Q_{O_2} and R.Q. values for rat-liver slices under various conditions

Eperjessy & Zathureczky (1947) gave R.Q. values of 0.92, 1.01 and 0.97 (twice) for 'control experiments' on liver slices from fasted rats during aerobic incubation in 0.067M-phosphate buffer, pH 7.2. The following average R.Q. values were also obtained by them in the presence of the substances mentioned (final concentrations shown in brackets): ethylamine hydrochloride, 0.53 (2×10^{-5} M); trimethylamine hydrochloride, 0.53 (4×10^{-5} M); tetramethylammonium iodide, 0.73 (2×10^{-5} M); choline chloride, 0.43 (2.5×10^{-5} M); ethanolamine hydrochloride, 0.63 (4×10^{-5} M); triethanolamine hydrochloride, 0.21 (2×10^{-5} M).

In the present work, the compounds listed earlier were tested under comparable conditions, control values without additions always being determined simultaneously. Table 1 indicates that in our experiments the control R.Q. values for liver slices from fasted rats all lay within the range 0.48–0.61, average 0.54. $-Q_{O_2}$ values varied from 6.2 to 9.0, average 7.2. When tested at 2×10^{-5} M, the amines, etc., were without effect on the R.Q. In view of this, the experiments were repeated using the amines at 2×10^{-2} M, since it was considered that the results of Eperjessy & Zathureczky might possibly have been caused by impurities present in their samples. Again, however, no systematic effect on the R.Q. was observed. With 2×10^{-2} M-choline chloride (Exp. 6), the O_2 consumption was raised above the control value, as was to be expected in view of the presence of an active choline oxidase in liver tissue; the R.Q. was also reduced. In all other cases, the amines were without effect on the rate of O_2 uptake. (For brevity, only the experiments with trimethylamine hydrochloride, choline chloride, ethanolamine hydrochloride and triethanolamine hydrochloride are quoted in Tables 1, 4 and 5; similar results were also

Table 1. *Respiration of liver slices from fasted rats in the presence and absence of some amines and related substances*

(*In vitro* respiration for 1 hr. in O₂ at 38°, pH 7.4.)

Exp. no.	Amine or related substance present				Controls	
		Concn. (M)	-Q _{O₂}	R.Q.	-Q _{O₂}	R.Q.
1	Trimethylamine HCl	2 × 10 ⁻⁵	8.70	0.54	7.76	0.57
2	Choline chloride	2 × 10 ⁻⁵	6.65	0.67	6.20	0.48
3	Ethanolamine HCl	2 × 10 ⁻⁵	8.59	0.54	8.95	0.54
4	Triethanolamine HCl	2 × 10 ⁻⁵	6.79	0.45	7.90	0.56
5	Trimethylamine HCl	2 × 10 ⁻²	5.75	0.51	6.25	0.50
6	Choline chloride	2 × 10 ⁻²	9.18	0.28	7.18	0.50
7	Ethanolamine HCl	2 × 10 ⁻²	5.55	0.64	6.20	0.61
8	Triethanolamine HCl	2 × 10 ⁻²	6.45	0.53	7.25	0.60

obtained with dimethylamine hydrochloride, triethylamine hydrochloride and tetramethylammonium iodide.)

In another series of experiments, the effect of triethanolamine hydrochloride on respiring liver slices from fasted rats was examined at several different concentrations. This substance was chosen for further investigation since Eperjessy & Zathureczky had found it to be more active than any other compound tested. The results shown in Table 2 indicate that at all concentrations used, triethanolamine hydrochloride was without significant effect on the R.Q. of liver slices from fasted rats.

Table 2. *Respiration of liver slices from fasted rats in the presence of different concentrations of triethanolamine hydrochloride*

(Experimental conditions as in Table 1.)

Exp. no.	Triethanolamine HCl present				Controls	
	Concn. (M)	-Q _{O₂}	R.Q.		-Q _{O₂}	R.Q.
9	5 × 10 ⁻⁶	8.11	0.47		6.05	0.35
10	2 × 10 ⁻⁵	6.58	0.54		7.25	0.60
11	2 × 10 ⁻⁴	7.45	0.67		6.65	0.42
12	2 × 10 ⁻³	8.06	0.55		6.00	0.34
13	2 × 10 ⁻²	7.99	0.61		6.66	0.47

The respiration medium used by Eperjessy & Zathureczky is given as 'm/15-phosphate buffer', pH 7.2, but the nature of the cations is not stated. In an attempt to find out whether the nature of the medium is important, some additional experiments were carried out using m/15 phosphate buffers of pH 7.2 having different cation compositions. The results using 2 × 10⁻⁵-m-triethanolamine hydrochloride are shown in Table 3; no effect on the R.Q. was seen.

Eperjessy & Zathureczky explained the lowering of the R.Q. of liver slices from fasted rats, in the presence of the substances used by them, as being due to two factors: (1) stimulation of fat metabolism, and (2) inhibition of carbohydrate metabolism. In order to provide support for this latter claim, they repeated some of their experiments with hexosediphosphate in the respiration medium.

The R.Q. of liver slices rose from the control value of 0.97 without HDP to an average value of 1.12 in the presence of 1.2 mg. HDP/ml. medium. The R.Q. values obtained when 10⁻⁵-m-amines, etc. were added to this HDP-containing system were as follows: ethylamine hydrochloride, 0.49; choline chloride, 0.59; triethanolamine hydrochloride, 0.21.

Table 3. *R.Q. of liver slices from fasted rats in presence and absence of 2 × 10⁻⁵-m-triethanolamine hydrochloride using phosphate buffers of different cation composition*

(All buffers were m/15 with respect to phosphate and of pH 7.2.)

Exp. no.	Triethanolamine HCl (2 × 10 ⁻⁵ -m) present		Controls		Cation composition of buffer (atoms %)
	-Q _{O₂}	R.Q.	-Q _{O₂}	R.Q.	
14	6.82	0.60	5.45	0.35	83 Na:17 K
15	4.92	0.44	6.40	0.59	100 K
16	6.48	0.32	5.93	0.33	100 Na

In the present work, the substances already mentioned have also been tested when HDP was present in the respiration medium. Table 4 shows that the R.Q. values, obtained in the presence of HDP only, lay in the range 0.40-0.56, average 0.49; -Q_{O₂} values varied from 5.9 to 8.7, average 7.2. Comparing these values with those given in Table 1, it appears that HDP had no effect on the respiration of liver slices from fasted rats. When tested at 2 × 10⁻⁵-m or 2 × 10⁻²-m, the compounds used had no effect upon the R.Q. values, except with 2 × 10⁻²-m-choline chloride (Exp. 22), the result being similar to that obtained in the absence of HDP.

Since only traces of glycogen are present in the livers of fasted rats, the extent of carbohydrate oxidation in such tissues is probably very slight. This would also be suggested by the low value of the R.Q. found in the experiments described. It has seemed, therefore, more logical to test for the inhibition of carbohydrate oxidation in the presence of the amines

Table 4. *Effect of some amines and related substances on the R.Q. of liver slices from fasted rats in the presence of hexosediphosphate*
(0.004M-NaHDP present in all vessels.)

Exp. no.	Amine or related substance present			Controls		
		Concn. (M)	$-Q_{O_2}$	R.Q.	$-Q_{O_2}$	R.Q.
17	Trimethylamine HCl	2×10^{-5}	7.19	0.44	6.91	0.40
18	Choline chloride	2×10^{-5}	8.12	0.58	8.32	0.51
19	Ethanolamine HCl	2×10^{-5}	6.01	0.38	5.96	0.40
20	Triethanolamine HCl	2×10^{-5}	6.45	0.44	7.70	0.52
21	Trimethylamine HCl	2×10^{-2}	7.58	0.52	8.71	0.56
22	Choline chloride	2×10^{-2}	10.20	0.28	6.93	0.54
23	Ethanolamine HCl	2×10^{-2}	6.83	0.56	6.54	0.50
24	Triethanolamine HCl	2×10^{-2}	6.96	0.64	6.67	0.46

Table 5. *Respiration of liver slices from well-fed rats in the presence and absence of certain amines and related substances*

Exp. no.	Amine or related substance present				Controls	
		Concn. (M)	$-Q_{O_2}$	R.Q.	$-Q_{O_2}$	R.Q.
25	Trimethylamine HCl	2×10^{-5}	10.79	0.87	10.64	0.78
26	Choline chloride	2×10^{-5}	8.66	0.75	8.35	0.85
27	Ethanolamine HCl	2×10^{-5}	8.57	0.78	9.16	0.77
28	Triethanolamine HCl	2×10^{-5}	7.73	0.73	6.71	0.77
29	Trimethylamine HCl	2×10^{-2}	9.69	0.73	10.89	0.80
30	Choline chloride	2×10^{-2}	10.09	0.37	7.82	0.79
31	Ethanolamine HCl	2×10^{-2}	9.82	0.79	10.95	0.83
32	Triethanolamine HCl	2×10^{-2}	8.39	0.69	9.01	0.68

Table 6. *Carbohydrate changes in rat-liver slices after incubation in the presence and absence of some amines and related substances*

(Total carbohydrate expressed as glucose, 1 hr. aerobic incubation for Exps. 33, 34 and 36; 1.5 hr. for others. In Exps. 38-40 well-fed rats were used, in remainder rats fasted 24 hr.)

Exp. no.	Amine or related substance used	Concn. (M)	Total carbohydrate (mg./g. fresh liver)			Method
			Initial value	Final value		
				Without amine	With amine	
33	Choline chloride	2×10^{-5}	3.36	4.32	4.21	Anthrone
34	Triethanolamine HCl	2×10^{-5}	6.92	8.68	8.01	Anthrone
35	Triethanolamine HCl	2×10^{-5}	4.34	5.36	5.96	Anthrone
			5.09	5.62	5.86	Anthrone*
			4.79	5.36	5.79	T.R.S.†
36	Dimethylamine HCl	2×10^{-2}	1.70	3.36	3.01	Anthrone
37	Triethylamine HCl	2×10^{-2}	2.24	3.96	3.46	Anthrone
38	Dimethylamine HCl	2×10^{-2}	59.6	57.2	50.7	T.R.S.†
39	Ethanolamine HCl	2×10^{-2}	55.5	54.6	53.3	Anthrone
40	Ethanolamine HCl	2×10^{-2}	46.6	46.2	45.8	Anthrone
			48.3	44.3	45.3	Anthrone*

* Total carbohydrate by anthrone method, after acid hydrolysis.

† Total reducing substances after acid hydrolysis.

and related substances by using liver slices from well-fed animals, in which carbohydrate oxidation is known to proceed. Accordingly, experiments similar to those already described have been carried out on liver slices from fed animals, in which the level of initial glycogen was approx. 4-6% (Table 5).

It will be observed that the control R.Q. values obtained for liver slices from fed rats were much higher than from fasted animals and lay in the range

0.68-0.85, average 0.78. Similarly, the $-Q_{O_2}$ values were also higher than for fasted rats, varying between 6.7 and 10.9, average 9.2. These figures are in excellent agreement with those of earlier workers (Jowett & Brooks, 1928; Dickens & Greville, 1933; Krebs, 1934; Gemmill & Holmes, 1935; Hastings, Muus & Bessey, 1939; Feinstein & Stare, 1940; Baernstein & Grand, 1941; Fuhrman & Field, 1944; Voigt, 1944; Stadie, Riggs & Haugaard, 1945). The

amines, etc., when present in $2 \times 10^{-5} \text{M}$ and $2 \times 10^{-2} \text{M}$ concentration are seen to be without effect on the R.Q. or Q_{O_2} values, except in the case of $2 \times 10^{-2} \text{M}$ -choline chloride. Thus these experiments afford no support for the view that carbohydrate oxidation in liver is inhibited by aliphatic amines and related compounds.

The carbohydrate content of rat-liver slices before and after incubation under different conditions

Since the R.Q. data provide no support, however indirect, for the claim of Eperjessy & Zathureczky (1947), that aliphatic amines inhibit carbohydrate oxidation in liver slices from fasted rats, it was decided to investigate this problem directly. To this end, carbohydrate determinations were made on liver slices from either fasted or fed animals before and after aerobic incubation in the presence and absence of added amines. Table 6 contains some representative data relating to the changes in total carbohydrate of rat-liver slices under these conditions. In all experiments using liver from fasted animals, there was a synthesis of carbohydrate from non-carbohydrate sources. Similar results were obtained by Bach & Holmes (1937) and Cross & Holmes (1937) for liver slices from fasted rats and rabbits respectively. The extent of this glycogenesis was not greatly influenced by the addition of the substances tested. When experiments were conducted with liver slices from well-fed animals, there was a slight utilization of carbohydrate. Table 6 indicates that the addition of aliphatic amines had little effect on the rate of carbohydrate utilization.

Effect of choline chloride on the respiration of liver slices from fasted rats

Choline chloride ($2 \times 10^{-2} \text{M}$) has already been shown to increase the $-Q_{O_2}$ and to reduce the R.Q. of liver slices from fasted rats (Table 1, Exp. 6), from fasted rats with *in vitro* addition of HDP (Table 4, Exp. 22) and from normally fed rats (Table 5, Exp. 30). These results might be due to stimulation of fat oxidation and simultaneous inhibition of carbohydrate oxidation by the choline added, but a more likely explanation is that choline behaves as a substrate, being oxidized to betaine aldehyde (Mann & Quastel, 1937), the overall reaction being given by

$$\text{Cl}^-[\text{N}^+(\text{CH}_3)_3 \cdot \text{CH}_2 \cdot \text{CH}_2\text{OH}] + \frac{1}{2}\text{O}_2 \rightarrow \text{Cl}^-[\text{N}^+(\text{CH}_3)_3 \cdot \text{CH}_2 \cdot \text{CHO}] + \text{H}_2\text{O}.$$

In order to determine whether or not choline oxidation does take place under the conditions of the experiment, Exp. 6 (Table 1) was repeated and choline was determined on duplicate samples treated with 0.2 ml. of 3N-hydrochloric acid at the beginning and end of the 1 hr. respiration period.

Table 7 presents the data obtained for the respiratory exchanges in this experiment; furthermore,

Table 7. *Choline oxidation by liver slices from a fasted rat*

(Exp. 41. Choline chloride (2.79 mg.) initially added to half of the Warburg flasks (final concn. $2 \times 10^{-2} \text{M}$). $-Q_{\text{choline}}$ expresses $\mu\text{l.}$ choline utilized/mg. dry wt. of tissue/hr., where $1 \mu\text{mol. choline} \equiv 22.4 \mu\text{l.}$)

	Choline present	Choline absent
R.Q.	0.33	0.56
$-Q_{O_2}$	9.58	7.90
Q_{CO_2}	3.16	4.43
$-Q_{\text{choline}}$	7.83	—

Increase in $-Q_{O_2}$ due to oxidation of choline to betaine aldehyde: calc., $\frac{1}{2}(7.83) = 3.92$; obs., $9.58 - 7.90 = 1.68$. Thus inhibition of endogenous $-Q_{O_2}$ by choline $= 3.92 - 1.68 = 2.24$. Inhibition of endogenous Q_{CO_2} by choline $= 4.43 - 3.16 = 1.27$. R.Q. of inhibited respiration $= 1.27/2.24 = 0.57$, which is similar to the R.Q. of the uninhibited endogenous respiration (0.56).

it shows that choline disappears under these conditions. From these results it also appears that choline slightly inhibits the endogenous respiration of liver slices from fasted rats, the O_2 uptake and CO_2 production being inhibited in the same ratio as that found for the endogenous R.Q. This type of inhibition is not, of course, of the kind required to support the hypothesis of Eperjessy & Zathureczky.

DISCUSSION

Attention has already been drawn to the discrepancy between the control Q_{O_2} and R.Q. values for liver slices from fasted rats obtained by Eperjessy & Zathureczky (1947) and those of other workers. Table 8 summarizes some of the earlier literature and includes the experiments carried out in the present study.

The Q_{O_2} values obtained in this laboratory lie in the lower part of the normal range found by other investigators. This may be due to a combination of three factors. First, enzyme deterioration may have taken place during the anaerobic storage of the accumulating liver slices before they were transferred to the Warburg vessels; this storage was necessitated by the cutting of about forty slices for each experiment. This deterioration was reduced, as far as possible, by keeping the slices in ice-cold medium during this period. Secondly, the respiration medium initially contained no bicarbonate, a constituent known to stimulate in marked degree the rate of oxygen consumption (Warren, 1944). Lastly the final dry weight of the tissue (23.1% of the moist weight), upon which our Q_{O_2} values depend, is greater than that found by some other workers. Thus the high Q_{O_2} values given by Elliott *et al.* (1937) are partly due to loss of material from their slices during incubation, since they obtained an average final dry weight of only 15.9% of the moist weight. However, in spite of these adverse factors, the Q_{O_2}

Table 8. Q_{O_2} and R.Q. values for liver slices from fasted rats(All Q_{O_2} values based on final dry weights. Phosphate-buffered media of different composition, pH 7.2-7.4, 37-38°.)

No. of exps.	$-Q_{O_2}$		R.Q.		References
	Average	Range	Average	Range	
22	8.9*	6.3-10.9	0.57	0.48-0.68	Bach & Holmes (1937)
7	8.2	$\pm 0.15^\dagger$	—	—	Craig (1943)
9	8.8	7.1-10.2	0.61	0.57-0.65	Dickens & Simer (1931)
13	11.4	9.4-13.7	—	—	Edson (1936)
3	9.1	4.9-12.7	0.49	0.46-0.53	Elliott & Baker (1935)
5	13.4‡	10.1-17.0	0.53	0.34-0.61	Elliott <i>et al.</i> (1937)
6	5.8§	$\pm 0.32^\dagger$	—	—	Fuhrman & Crismon (1944)
6	8.7	$\pm 0.18^\dagger$	—	—	Fuhrman & Crismon (1944)
25	6.8	5.1-9.0	0.51	0.33-0.66	Present work
4	3.6*	3.1-3.9	0.97	0.92-1.01	Eperjessy & Zathureczky (1947)

* Calculated from values quoted for fresh moist weight of tissue. Final dry weight of 23.1% assumed.

† S.D. of mean value.

‡ High value partly due to very low percentage dry weight (average 15.9%).

§ High potassium, chloride-free 'intracellular' medium.

values obtained in the present work are still nearly twice as great as those found by Eperjessy & Zathureczky. No other such low values have been found in the literature.

In a similar way, the control R.Q. values of Eperjessy & Zathureczky are much higher than those found by other workers. Their values, being of the order of unity, are nearly twice as high as those found in the present work, which however are in complete agreement with those given in the literature. R.Q. values of about 0.50 would seem to be reasonable for fasted liver slices, for apart from any question of incomplete oxidation of fatty acids, glyconeogenesis would also have the effect of lowering the R.Q. It is thus very difficult to see how these high values of Eperjessy & Zathureczky could have been obtained for liver slices from fasted rats. Hindemith (1942) has shown that the R.Q. of rat-liver slices is dependent on the glycogen content, his values for stock animals lying between 0.58 (low liver glycogen) and 0.85. Other workers have generally obtained figures of about 0.80 for normally fed rats and similar figures have been obtained in the present study. Only Elliott & Baker (1935), using stock rats fed a fairly high carbohydrate diet, appear to have obtained R.Q. values for liver slices approaching unity, the average figure being 0.99 with a range of 0.71-1.07. These workers drew attention to their high R.Q. values, as compared with other published data, but could offer no explanation.

The basic claim of Eperjessy & Zathureczky is that liver slices from fasted rats show a lowering of the R.Q. without change in oxygen consumption in the presence of aliphatic amines. However, even if this claim could be substantiated, their interpretation in terms of a simultaneous stimulation of fatty acid oxidation and inhibition of carbohydrate oxidation would need to be confirmed by further experimental evidence. The determinations of carbohydrate

changes, given in the experimental part of this paper, have provided no support for this hypothesis.

Choline is the only substance tested which has been found to be oxidized by rat liver slices when used at substrate concentration ($2 \times 10^{-2} M$). The closely related primary alcoholic compounds, ethanolamine and triethanolamine, had no effect upon the Q_{O_2} value. In addition to undergoing oxidation, choline had a slight inhibitory effect on the endogenous respiration of rat liver. Thus in the experiment quoted, it was calculated that for oxidation to betaine aldehyde of the choline disappearing, it was necessary to assume 28% inhibition of the endogenous oxygen uptake; the observed inhibition of CO_2 production in the presence of choline was 29%. In other words, the various oxidation processes concurrently taking place in respiring rat-liver slices are inhibited non-selectively by choline.

SUMMARY

1. Liver slices from fasted rats, respiring in oxygen at 38° in a phosphate-buffered medium, pH 7.4, showed an average $-Q_{O_2}$ of $6.8 \mu l./mg.$ dry wt./hr. and an average R.Q. of 0.51. These values are in general agreement with those reported in the literature.

2. A number of aliphatic amines and related substances, viz. choline chloride, tetramethylammonium iodide and the hydrochlorides of dimethylamine, trimethylamine, triethylamine, ethanolamine and triethanolamine, when tested in 2×10^{-5} and $2 \times 10^{-2} M$ concentrations, were found to be without effect on either the Q_{O_2} or R.Q. values. The latter result contrasts with the claim of Eperjessy & Zathureczky (1947), who observed a substantial lowering of the R.Q.

3. The same compounds were without effect on carbohydrate utilization by liver slices from well-fed

rats or on glyconeogenesis by liver slices from fasted rats.

4. Of the substances mentioned, only choline chloride was oxidized by rat-liver slices. $2 \times 10^{-2} \text{M}$.

Choline chloride also inhibited endogenous respiration by about 30 %.

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The Reaction of Oxidizing Agents with Wool

1. THE DIVISION OF CYSTINE INTO TWO FRACTIONS OF WIDELY DIFFERING REACTIVITIES

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Most processes for preventing the felting of wool make use of strong oxidizing agents (Speakman, Nilssen & Elliott, 1938; Freney, 1946), and the reaction of wool with these reagents is thus of technical as well as of scientific interest. The disulphide bond of cystine is one of the most reactive centres in the wool structure and it is therefore not surprising that oxidizing agents act preferentially upon it. Smith & Harris (1936) showed that hydrogen peroxide oxidizes the disulphide bond in wool slowly, whereas fluorine (Hudson & Alexander, 1946) reacts rapidly and in the initial stages specifically. Consden, Gordon & Martin (1946) identified cysteic acid by paper chromatography in the products of reaction between chlorine or bromine and wool. Speakman (1947) deduced from changes in the mechanical properties

of individual fibres, assuming that cystine cross-links the polypeptide chains, that the disulphide bond is attacked by many oxidizing agents.

Several workers have observed that the disulphide bond of cystine in wool exhibits varying reactivities. Phillips and his co-workers showed in a series of detailed papers (summarized by Phillips, 1946) that the cystine in wool can be divided into four fractions, which differ from one another both in the reaction products formed and in the rate of reaction on treatment with alkali, formaldehyde, bisulphite and thiolacetic acid. Elöd & Zahn (1947) found that even after a substantial proportion of the disulphide bonds had been broken the mechanical properties remained almost unaltered. On the basis of this evidence he proposed that the cystine is incorporated