was compared with that appearing in supernatants of experiments with cell suspensions of *E. coli* 7–8, a mutant known to produce indole-3-glycerol[3,11].

The tests set out in Table I indicate some of the similarities between the compound formed by *L. plantarum* and indole-3-glycerol formed by *E. coli* 7–8.

Supernatants from experiments with *L. plantarum* and *E. coli* were chromatographed in methanol–butanol–benzene–water (2:1:1:1) and the papers sprayed with Ehrlich's reagent. In both cases purple spots, $R_F$ 0.33, were obtained after heating.

The spectrum of the compound formed by *L. plantarum* had the characteristic form of that given by indole-3-glycerol, with peaks at 278 and 287 mμ (refs. 3, 11).

Further evidence was obtained which indicated that indole-3-glycerol phosphate did not accumulate in the cell suspensions of *L. plantarum*. Thus incubation of experimental samples with crude extracts of *E. coli* possessing high tryptophan synthetase activity and with serine and pyridoxal phosphate showed that the compound previously identified as indole-3-glycerol was not removed. Indole-3-glycerol phosphate would have been converted to tryptophan[15].

It seems therefore that the compound formed by cell suspensions of *L. plantarum* from indole or anthranilic acid is probably indole-3-glycerol.

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## The stimulation of the phosphogluconate oxidation pathway by pyruvate under anaerobic conditions in diaphragm muscle and in rat brown adipose tissue

It is generally accepted that glucose metabolism in diaphragm muscle proceeds solely by way of the glycolytic pathway; experiments carried out with specifically labelled [$^{14}$C]glucose have given no evidence for the occurrence of the phosphogluconate

oxidative pathway in muscle[1,2]. In the present work, however, it has been shown that the enzymes involved in the decarboxylation of glucose via the phosphogluconate pathway are in fact present, and their activity can be demonstrated by incubating the muscle anaerobically in the presence of [1-$^{14}$C]glucose and pyruvate. This effect of pyruvate has been described by KINOSHITA[3] in experiments carried out on corneal epithelium and have been interpreted by him as due to the reoxidation of the TPNH formed in the reactions of the phosphogluconate pathway of glucose metabolism, by the enzyme lactic acid dehydrogenase in the presence of pyruvate.

In the present experiments approx. 100 mg of diaphragm muscle from male Wistar rats (weighing from 100–200 g) were incubated in small Warburg vessels under the experimental conditions given in Table I. The substrate was added from the side-arm of the Warburg vessels after they had been gassed for 5 min with pure $N_2$. At the end of the incubation period the $CO_2$ was collected and the $^{14}$C content measured as previously described[4].

The results given in Table I show that pyruvate greatly stimulated the formation of $^{14}CO_2$ from [1-$^{14}$C]glucose. In similar experiments carried out with [6-$^{14}$C]glucose there were only traces of $^{14}CO_2$ formed either in the absence or presence of pyruvate.

The influence of insulin on the hexose monophosphate pathway of glucose metabolism has been shown in mammary gland tissue[5], in white adipose tissue[6,7] and by the present authors in brown adipose tissue (unpublished results). In muscle, however, there is no indication that this pathway of glucose metabolism functions under aerobic conditions, either in the absence or presence of insulin, as shown by the results given in Table II. It is conceivable, however, that under aerobic conditions

TABLE I

INFLUENCE OF PYRUVATE ON THE ANAEROBIC $^{14}CO_2$ FORMATION FROM [1-$^{14}$C]GLUCOSE IN THE ISOLATED RAT DIAPHRAGM

Results expressed as total counts/min of $^{14}CO_2$ formed after incubation of 100 mg tissue in 1 ml of Krebs–Ringer phosphate medium for 1 h at 37° in $N_2$. Glucose concentration, 0.1%; 1 $\mu$C [1-$^{14}$C]glucose per vessel, specific activity 1.6 $\mu$C/mg. Mean values of 7 experiments given ± standard error of mean.

| *No pyruvate* | *With pyruvate (20 mM)* |
|---|---|
| 540 ± 60 | 1510 ± 110 |

TABLE II

AEROBIC FORMATION OF $^{14}CO_2$ FROM [1-$^{14}$C]GLUCOSE AND [6-$^{14}$C]GLUCOSE IN THE ISOLATED RAT DIAPHRAGM IN THE ABSENCE AND PRESENCE OF INSULIN

Results expressed as total counts/min of $^{14}CO_2$ formed after incubation of 100 mg of tissue in 1 ml of Krebs–Ringer phosphate medium for 1 h at 37° in $O_2$. Glucose concentration, 0.1%; 1 $\mu$C of [1-$^{14}$C]glucose or [6-$^{14}$C]glucose per vessel, specific activity, 1.6 $\mu$C/mg; insulin concentration, 0.1 unit/ml. Mean value of 12 experiments given ± standard error of mean.

| *[1-$^{14}$C]glucose* | | *[6-$^{14}$C]glucose* | |
|---|---|---|---|
| *No insulin* | *With insulin* | *No insulin* | *With insulin* |
| 7200 ± 540 | 7300 ± 580 | 6500 ± 490 | 7200 ± 590 |

TABLE III

INFLUENCE OF PYRUVATE ON THE ANAEROBIC $^{14}CO_2$ FORMATION FROM [1-$^{14}$C]GLUCOSE IN BROWN ADIPOSE TISSUE SLICES

Results expressed as total counts/min of $^{14}CO_2$ formed after incubation of 100 mg tissue in 1 ml of medium containing 0.1% glucose at 37° in $N_2$ or in 95% $N_2$–5% $CO_2$; 1 $\mu$C [1-$^{14}$C]glucose per vessel.

| *Expt.* | *Medium* | *Time of incubation (min)* | *No pyruvate* | *With pyruvate (20 mM)* |
|---|---|---|---|---|
| 1 | Krebs–Ringer bicarbonate | 30 | 490 | 1980 |
| | | 60 | 530 | 2880 |
| | | 120 | 890 | 4510 |
| | | 150 | 2490 | 8610 |
| 2 | Krebs–Ringer phosphate | 30 | 290 | 1530 |
| | | 60 | 420 | 3400 |
| | | 120 | 910 | 7200 |
| | | 150 | 1150 | 13100 |

the active glycolytic pathway of glucose oxidation masks the less active hexose monophosphate pathway, which can thus no longer be brought into evidence by the available techniques.

A similar effect of pyruvate on the phosphogluconate oxidative pathway under anaerobic conditions was also shown in slices of brown adipose tissue. The slices were prepared as previously described[8] and incubated in a Krebs–Ringer phosphate or Krebs–Ringer bicarbonate medium for varying time periods under the experimental conditions given in Table III. In the bicarbonate medium the gas phase was 95% $N_2$–5% $CO_2$ and the $CO_2$ was liberated from the medium at the end of the incubation with acid and collected as previously described[9].

The results given in Table III show that in this tissue also there was a considerable stimulatory effect of pyruvate on $^{14}CO_2$ formation at all the time periods measured in both media.

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