man & Vilee, 1959; Jarabak, Adams, Williams-Ashman & Talalay, 1962). Talalay & Williams-Ashman (1958) suggested that a steroid dehydrogenase with dual nucleotide specificity is responsible for steroid-dependent transhydrogenation, with one site on the enzyme for which both nucleotides compete. At the stage of enzyme purification reached in the present work it has not been possible to separate NAD- or NADP-linked 3α -hydroxy steroid-dehydrogenase activities from the transhydrogenase activity. It has been found, however, that ammonium sulphate stimulated the NAD-linked dehydrogenase but not the NADP-linked dehydrogenase activity.

Until the mechanism of 3α -hydroxy steroid-dependent transhydrogenation in rat liver is more fully explained by work with purer enzyme preparations, the role of anions in these reactions will be difficult to define.

SUMMARY

1. 3α -Hydroxy steroid-dependent nicotinamide-adenine dinucleotide transhydrogenase from rat liver, partially purified by ammonium sulphate fractionation and column chromatography on diethylaminoethylcellulose, is activated by some bivalent anions.

2. The effect of SO_4^{2-} ions on the optimum H^+ ion, steroid and NADP concentrations of the

system, and on the reversibility of the reaction, have been examined.

3. Some effects of the SO_4^{2-} ions on NAD- and NADP-linked androsterone-dehydrogenase activity have also been studied.

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Tissue Concentrations of Coenzyme Q, Ubichromenol and Tocopherol in Relation to Protein Status in the Rat

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Aromatic biosynthesis, except for partial synthesis of the steroid nucleus in the oestrogens, is known not to occur in animal tissues. Therefore it was considered that coenzyme Q (ubiquinone), a benzoquinone, may in some measure be a vitamin (Morton, 1958). However, a state of deficiency in coenzyme Q could not be produced in rats by any of the known synthetic diets (Lawson, Threlfall, Glover & Morton, 1961; Olson, Dialameh & Bentley, 1961). Further, several lines of evidence (summarized by Ramasarma, 1961) rule out the possibility of intestinal bacteria being the source of coenzyme Q. Olson *et al.* (1961) showed that uniformly labelled phenylalanine was incorporated

into coenzyme Q and established that phenylalanine serves as the precursor of the quinone nucleus. It is logical therefore to expect that changes in the concentration of dietary protein, and consequently of phenylalanine, will affect the amounts of coenzyme Q in the tissues.

EXPERIMENTAL

The diet contained 5% of groundnut oil, 5% of salt mixture (U.S. Pharmacopeia, 1947), casein in the percentage mentioned in the text, 10% of cane sugar and starch to 100%. Each rat received 0.5 ml. of vitamin B-complex solution (U.S. Pharmacopeia, 1947)/day. Vitamin A

(250 $\mu\text{g.}$), vitamin D (1.5 $\mu\text{g.}$), tocopherol (2 mg.) and vitamin K (0.05 mg.) were given to each rat weekly. Water was supplied *ad libitum*. The diet contained 90 μm -moles of coenzyme Q/100 g.; the major part of this is present in the groundnut oil, so that all the diets, irrespective of their protein contents, have nearly equal quantities of coenzyme Q. The rats were kept in galvanized-iron cages (two per cage), and there was no possibility of adventitious protein or coenzyme Q becoming available for consumption.

In the first set of experiments, 12 adult female albino rats, each weighing about 150 g., were used. They were divided into two groups of six rats each, one group receiving the diet without protein, and the second group receiving the diet with 20% of protein, for 3 weeks.

In the second set of experiments, weanling albino rats of both sex, each weighing about 50 g., were employed. Four groups containing six rats in each were fed *ad libitum* with protein-free diet. One group was killed in the beginning and the other three groups at the end of 10, 20 and 30 days respectively.

In the third set of experiments, weanling albino rats of both sex, each weighing about 45 g., were employed. Six groups containing six rats in each were fed *ad libitum*, the diet containing 0, 6, 12 or 20% of casein, for 3 weeks. Two groups received (a) DL- β -phenylalanine (50 mg./rat/day) or (b) coenzyme Q₁₀ (0.3 mg./rat on alternate days) with the diet containing 6% of protein.

At the end of the experimental period the animals were killed after ether anaesthesia. Liver, heart, kidney, spleen and brain were removed and weighed. Since a large number of samples were involved organs of the same group were pooled for analysis as recommended by Edwin, Diplock, Bunyan & Green (1961). It has been shown by these authors and confirmed in this Laboratory that such pooled analyses are statistically as significant as the analyses made with individual organs.

Portions of the liver were removed from each group and 10% homogenates were prepared in 0.25 M-sucrose solution with a Potter-Elvehjem-type glass homogenizer. Cell fractions were obtained by the conventional differential fractionation procedure of Schneider & Hogeboom (1950).

Protein in all the aqueous homogenates as well as in the mitochondrial fractions (with added deoxycholate; 0.1 ml, 1%) was estimated by the biuret method (Gornall, Bardawill & David, 1949).

Succinoxidase activity was measured manometrically according to Schneider & Potter (1943).

Homogenates (10 ml., 10%) of organs were saponified under reflux with 1 ml. of 40% sodium hydroxide in 75% ethanol, in the presence of 1 ml. of ethanolic 5% pyrogallol, for 30 min. Coenzyme Q, ubiquinone and tocopherol in the unsaponifiable matter were separated by the use of the reverse-phase paper-chromatographic system of Lester & Ramasarma (1959). The spots corresponding to coenzyme

Q, ubiquinone and tocopherol were cut out and extracted with 3 ml. of chloroform followed by 3 ml. of light petroleum (b.p. 40–60°), and the compounds were estimated by the bipyridyl–ferric chloride method (Jayaraman & Ramasarma, 1961).

[¹⁴C]Coenzyme Q₁₀, prepared by chemical synthesis, labelled in the first two carbon atoms of the side chain shown by asterisks in Fig. 1 (specific activity 10 $\mu\text{C}/\text{mg.}$), was used. The labelled compound (1.0 μC , as aqueous emulsion) was given orally to two rats each in the 0, 6, 12 and 20% protein dietary groups. After 12 hr. the rats were killed after ether anaesthesia and the livers removed for analysis. The livers were saponified and the radioactivity in the unsaponifiable matter was measured. All the samples were plated on aluminium planchets and counted in the Geiger counter (Nuclear-Chicago Corp. model 151A) and the counts were corrected for self-absorption and back-ground.

RESULTS

Protein status and coenzyme Q. Deprivation of protein in the diet resulted in lowering the amounts of coenzyme Q and ubiquinone in only two organs of the adult rat: liver and heart (Table 1). The two compounds decreased in content expressed either as μm -moles/g. of tissue or as μm -moles/whole organ, whereas the average weights of organs had not changed to any large extent. Other organs tested, kidney, spleen and brain, contained small concentrations of coenzyme Q and ubiquinone and the changes were insignificant. Tocopherol contents of the tissues suffered no marked changes.

In the second set of experiments it was observed that on protein deprivation the amounts of coenzyme Q, ubiquinone and tocopherol in liver and heart decreased to a considerable extent after 10 days, and there were no marked changes in coenzyme Q content during other time-intervals (Table 2). In this set of experiments, the individual liver samples were analysed for coenzyme Q content and the values of means and standard deviations are given in Table 2. These agree with the values of analysis of pooled samples and confirm the decrease in coenzyme Q contents of livers during protein starvation.

In the third set of experiments weanling rats were used and the scope was extended by comparing the percentage of protein in the diet in relation to the contents of coenzyme Q, ubi-

Fig. 1. Coenzyme Q₁₀. Asterisks indicate the labelled carbon atoms.

chromenol and tocopherol in the organs (Table 3). The contents of coenzyme Q and ubiquinomenol increased in liver and heart with the increasing percentages of protein in the diet fed to rats. The 'initial' amounts, analysed in a group before the start of the experiment, were nearly maintained with minimal (6%) protein in the diet, but with complete deprivation of protein these amounts decreased. No significant changes were found in kidney, spleen and brain. Tocopherol content was high in livers of animals fed on greater amounts of protein. Random variations in the tocopherol content of kidney, spleen and brain could not be correlated with protein status of the rat.

Protein status and succinoxidase activity. Since the coenzyme Q content in the liver was lowered on protein deprivation, it was investigated whether lowering of the succinoxidase activity of liver also occurred. The results in Table 4 show that the succinoxidase activity of liver homogenates and mitochondria increased progressively with the

increase in the dietary protein concentration, and this also corresponds to an increase in concentration of coenzyme Q in liver. These results are in support of the established role for coenzyme Q in electron transport (Green, D. E., 1961).

Effect of supplementation with phenylalanine and coenzyme Q₁₀ in the protein-deficient diet. Additional groups of weanling rats, in the second set of experiments, were kept on 6% protein diet supplemented with phenylalanine (50 mg./rat/day) in one group and with coenzyme Q (0.3 mg./rat on alternate days) in another. With 6% of protein in the diet the necessary enzymes for biosynthesis of coenzyme Q would probably be available and the additional phenylalanine may therefore be expected to supply the benzenoid precursor of the quinone nucleus of coenzyme Q. In agreement with this hypothesis, the coenzyme Q and ubiquinomenol contents have increased, in liver, heart and kidney, on supplementing with phenylalanine (Table 3). The most marked increase was observed in the ubi-

Table 1. *Effect of protein deprivation on the amounts of coenzyme Q, ubiquinomenol and tocopherol in organs of the adult rat*

Organ	Protein in diet (%)	Mean wt. of organ (g.)	Coenzyme Q		Ubiquinomenol		Tocopherol	
			(μ m-moles/g.)	(μ m-moles/organ)	(μ m-moles/g.)	(μ m-moles/organ)	(μ m-moles/g.)	(μ m-moles/organ)
Liver	0	6.70	115.0	770.0	2.3	15.5	3.5	24.4
	20	6.11	167.0	1020.0	24.9	152.0	2.8	17.0
Heart	0	0.49	105.0	51.7	11.5	5.6	5.6	2.7
	20	0.56	139.0	78.0	16.0	8.9	16.5	9.3
Kidney	0	0.90	45.3	40.8	7.1	6.4	5.1	4.6
	20	1.23	37.1	45.6	7.2	8.8	5.3	6.6
Spleen	0	0.53	2.8	1.5	24.8	13.1	8.6	4.6
	20	0.90	1.7	1.6	4.6	4.2	6.0	5.4
Brain	0	1.44	3.5	5.0	1.6	2.3	4.6	6.7
	20	1.37	5.9	8.1	1.7	2.4	4.4	6.0

Table 2. *Amounts of coenzyme Q, ubiquinomenol and tocopherol in weanling rat tissues during protein deprivation*

Tissue	Period of protein deprivation (days)	Mean wt. of organ (g.)	Coenzyme Q		Ubiquinomenol		Tocopherol	
			(μ m-moles/g.)*	(μ m-moles/organ)	(μ m-moles/g.)	(μ m-moles/organ)	(μ m-moles/g.)	(μ m-moles/organ)
Liver	Initial	3.02	186.0	615.0	18.3	55.3	13.4	40.5
	10	3.33	(175 $\pm$ 4.8)	294.0	11.3	37.6	6.5	21.6
	20	2.52	(82 $\pm$ 4.9)	191.0	10.7	27.0	3.6	9.1
	30	2.57	(76 $\pm$ 3.6)	228.0	10.7	27.5	8.6	22.1
			(89 $\pm$ 6.4)					
Heart	Initial	0.24	230.0	55.2	9.9	2.4	25.7	6.2
	10	0.27	175.0	47.3	7.1	1.9	14.0	3.8
	20	0.23	155.0	35.7	4.7	1.1	10.2	2.3
	30	0.24	151.0	36.2	—	—	—	—

* Obtained by analysis of pooled samples; values in parentheses are the means and standard deviations of individual analyses of six liver samples.

Table 3. *Dietary protein status and tissue contents of coenzyme Q, ubiquinomenol and tocopherol in weanling rats*In the 6% protein diet (a) included DL- β -phenylalanine (50 mg./rat/day) and (b) included coenzyme Q₁₀ (0.3 mg./rat on alternate days).

Organ	Protein in diet (%)	Mean wt. of organ (g.)	Protein in tissue		Coenzyme Q		Ubichromenol		Tocopherol	
			(mg./g.)	(mg./organ)	(μ m-moles/g.)	(μ m-moles/ organ)	(μ m-moles/g.)	(μ m-moles/ organ)	(μ m-moles/g.)	(μ m-moles/ organ)
Liver	Initial*	3.09	217	669	29.0	90.0	16.0	49.3	16.5	51.0
	0	2.45	195	478	11.1	27.4	19.7	48.2	16.6	40.7
	6	3.96	130	515	27.8	110.0	37.6	148.0	26.3	104.0
	12	5.73	198	1135	47.5	274.0	47.0	270.0	32.1	133.5
	20	5.06	223	1130	90.0	454.0	45.2	228.0	26.0	132.0
Heart	6 (a)	5.04	200	1008	38.2	196.0	41.2	208.0	26.0	131.0
	(b)	4.08	191	780	34.5	141.5	33.8	138.0	29.8	123.0
	Initial*	0.22	210	46	73.0	16.1	21.3	4.6	16.7	3.7
	0	0.23	181	41	66.5	15.1	11.4	2.6	10.0	2.3
	6	0.28	198	56	51.0	14.4	21.1	5.9	16.3	4.6
Kidney	12	0.38	156	60	—	—	—	—	—	—
	20	0.40	190	77	97.4	39.4	26.8	10.8	16.0	6.5
	6 (a)	0.34	187	64	70.0	24.1	23.4	8.1	7.0	2.4
	(b)	0.32	177	55	31.5	9.9	21.4	6.7	10.6	3.3
	Initial*	0.70	176	123	7.0	4.9	28.0	19.7	5.4	3.7
Spleen	0	0.59	148	87	23.4	13.8	17.8	10.5	22.1	13.0
	6	0.62	163	101	31.6	19.6	11.8	7.3	12.8	7.9
	12	0.97	134	128	27.8	26.9	11.0	10.6	8.9	8.6
	20	1.16	128	147	31.6	36.6	13.5	15.6	16.5	19.0
	6 (a)	0.75	167	124	31.6	23.5	21.4	16.0	41.2	30.7
Brain	(b)	0.76	121	92	42.1	31.8	14.9	11.8	16.0	12.1
	Initial*	0.23	260	60	8.5	2.0	13.9	3.2	6.1	1.4
	0	0.15	166	25	11.1	1.7	19.0	2.9	19.5	3.0
	6	0.44	252	111	4.8	2.1	6.3	2.8	19.0	8.4
	12	0.78	180	141	—	—	—	—	9.8	7.7
Brain	20	0.92	175	161	—	—	11.6	10.6	17.3	15.9
	6 (a)	0.57	280	160	5.1	2.9	9.0	5.2	—	—
	(b)	0.36	198	72	2.6	1.5	20.4	7.4	18.1	6.5
	Initial*	1.04	119	124	8.5	8.9	16.9	17.6	4.4	4.7
	0	1.08	115	123	9.4	10.1	4.3	4.6	13.5	14.5
Brain	6	1.05	110	116	12.7	13.3	4.3	4.5	6.1	6.4
	12	1.13	104	118	4.4	5.0	9.6	10.9	8.2	9.3
	20	1.06	104	109	4.4	4.7	9.8	10.9	16.0	16.9
	6 (a)	1.12	119	134	12.7	14.5	6.7	7.5	7.2	8.1
	(b)	1.06	105	112	29.4	31.4	10.7	11.3	10.7	11.4

* Initial values were obtained from one group of animals at the beginning of the experiment.

chromenol content of the liver. Spleen and brain showed no significant changes. The succinoxidase, however, showed no increase in mitochondria and, in fact, decreased in the homogenate.

Supplementation with coenzyme Q₁₀ in the 6 % protein diet resulted in increased concentration of coenzyme Q in liver, kidney and brain (Table 3). Lawson *et al.* (1961) found that dietary coenzyme Q was absorbed and deposited primarily in the liver. This finding has been confirmed with [¹⁴C]-coenzyme Q₁₀ (Jayaraman, Joshi & Ramasarma, 1963). After oral administration [¹⁴C]coenzyme Q₁₀ was found, to a large extent, in liver but not in other organs tested, including brain, which is shown to contain coenzyme Q₁₀ as the predominant form (Green, J., 1961). In view of this the present observation of higher content of coenzyme Q in brain is interesting and probably

represents a stimulation of local synthesis. Further, added coenzyme Q decreased the succinoxidase activity in homogenate as well as in mitochondria.

Distribution of protein and coenzyme Q in liver-cell fractions. The results presented in Table 3 show that the amount of protein/g. of liver is altered only slightly with the increase in the percentage of protein in the diet whereas coenzyme Q content increased progressively. From the data presented in Table 5 it is evident that the distribution pattern of protein and coenzyme Q in the liver-cell fractions remained nearly the same irrespective of the content of coenzyme Q in the liver and content of protein in the diet.

Absorption of coenzyme Q₁₀ in relation to protein status. Orally administered [¹⁴C]coenzyme Q₁₀ was absorbed by the liver to the extent of 5-6 % in normal rats in 12 hr. This absorption capacity

Table 4. Succinoxidase activity in rat-liver homogenates and mitochondria

In the 6 % protein diet (a) included DL-β-phenylalanine (50 mg./rat/day) and (b) included coenzyme Q₁₀ (0.3 mg./rat on alternate days). Succinoxidase activity was measured in the Warburg manometer at 30°. One unit is taken to be 1.0 μl. of oxygen absorbed/hr.

Protein in diet (%)	Protein (mg./g. of tissue)	Succinoxidase activity				
		Homogenate			Mitochondria	
		(units/mg. of protein)	(units/g. of tissue)	(units/μm-mole of coenzyme Q)	(units/mg. of protein)	(units/μm-mole of coenzyme Q)
0	195	13.0	2535	229	36.5	146
6	130	22.7	2951	106	30.4	78
12	198	25.0	4950	104	52.8	77
20	223	43.2	9634	107	75.0	84
6 (a)	200	13.2	2640	68	31.2	—
(b)	191	16.5	3150	91	18.2	—

Table 5. Intracellular distribution of protein and coenzyme Q in rat-liver-cell fractions

The values were obtained in fractions from 1 g. of liver. The total in the four fractions is taken as 100 %. N, Nuclear fraction; Mt, mitochondrial fraction; Mc, microsomal fraction; St, supernatant.

Protein in diet (%)	Fraction	Protein		Coenzyme Q	
		(mg./g. of liver)	(%)	(μm-moles/g. of liver)	(%)
0	N	46.0	28.0	3.0	23.0
	Mt	24.0	14.6	6.0	46.0
	Mc	34.5	21.0	2.0	15.4
	St	59.8	36.4	2.0	15.4
6	N	35.0	23.0	5.5	25.5
	Mt	23.0	18.4	9.0	42.0
	Mc	24.0	19.2	4.5	21.0
	St	43.0	24.4	2.5	11.6
12	N	42.8	24.1	9.2	22.5
	Mt	21.9	12.3	15.0	36.8
	Mc	38.0	22.4	7.5	18.4
	St	75.0	41.2	9.1	22.3
20	N	53.0	25.5	24.0	30.5
	Mt	28.8	13.8	25.5	34.0
	Mc	40.0	19.2	14.2	17.9
	St	85.5	41.5	13.8	17.6

Table 6. Absorption of [^{14}C]coenzyme Q_{10} by protein-deficient rats

Average values from livers of two rats are given in each case. Each rat received $1.0\ \mu\text{C}$ of [^{14}C]coenzyme Q_{10} orally, and was killed after 12 hr.

Protein in diet (%)	Wt. of liver (g.)	Counts/min./ organ
0	2.27	2890
6	2.64	3410
12	5.67	5830
20	7.03	5500

decreased in rats fed on a low protein diet (Table 6). From the data it is clear that protein helps in the absorption of coenzyme Q.

DISCUSSION

The syndrome of protein deficiency is characterized by loss of many functions and involves several secondary effects in the animal. These could be directly related to the lack of protein or its constituents, resulting either in derangement in enzyme synthesis or the synthesis of essential metabolites or both. It is possible therefore that supplementing with some of these metabolites might help in partial alleviation of the deranged functions.

Coenzyme Q is known to participate in electron transport and possibly oxidative phosphorylation (Green, D. E., 1961). The coenzyme Q content in liver decreased on protein deprivation and this effect is more marked in weanling than adult rats. This could have been due to a decrease in the synthesizing enzymes or due to lack of phenylalanine, the precursor for the quinone nucleus. It appears that both the possibilities contribute to the decreased coenzyme Q content since supplementation with phenylalanine increased the amount, but not completely.

The succinoxidase activity decreased considerably in the livers of rats fed on a low percentage of protein and this correlated with the decrease in the coenzyme Q content of the livers. Supplementing the diet containing 6% of protein with coenzyme Q increased liver coenzyme Q content but not the succinoxidase activity. This could be due to either the lack of the enzymes or the inability of extra coenzyme Q to reach the active site. The distribution of coenzyme Q in liver-cell fractions was unaffected at different liver coenzyme Q concentrations. [^{14}C]Coenzyme Q_{10} fed in the diet was deposited in the liver and was found to be distributed in the cell fractions, including mitochondria, in nearly the same proportion as the non-radio-

active compound (J. Jayaraman & T. Ramasarma, unpublished work). It seems therefore that the absence of effect of extra coenzyme Q on succinoxidase is probably due to the lack of additional quantities of enzymes in proportion to coenzyme Q. This is also supported by the data on the specific activities of succinoxidase, expressed as units/ $\mu\text{m-mole}$ of coenzyme Q, which remained fairly constant in the groups fed on 6, 12 and 20% of protein.

We have observed that orally-administered [^{14}C]mevalonic acid was incorporated into ubiquinomenol and coenzyme Q in rat liver and that the specific activity in ubiquinomenol was higher, suggesting that it may be a precursor of coenzyme Q (V. C. Joshi, J. Jayaraman & T. Ramasarma, unpublished work). The amounts of ubiquinomenol, which accompanies coenzyme Q in animal tissues, also showed corresponding increases, particularly in liver, with high-protein diets and with added phenylalanine, but not with added coenzyme Q_{10} . These results are in support of the view that ubiquinomenol is a precursor of coenzyme Q.

Under the stress condition of protein deficiency, the animal, although showing decreased growth in total body weight and weight of individual tissues, appears to conserve minimal protein and coenzyme Q contents in the essential organs, particularly liver, and also maintains the important energy-yielding reactions to help survival.

SUMMARY

1. Protein deprivation in the diet of adult and weanling rats resulted in lowering the contents of coenzyme Q and ubiquinomenol in liver and heart.
2. The contents of coenzyme Q and ubiquinomenol in liver and heart of weanling rats increased with the increase in the percentage of protein in the diet. The coenzyme Q contents of kidney, spleen and brain were little affected.
3. Addition of phenylalanine or coenzyme Q to the diet containing 6% of casein increased the coenzyme Q content in the liver.
4. The liver-succinoxidase activity markedly decreased as the protein concentration in the diet decreased. Addition of coenzyme Q or phenylalanine to the diet with 6% of casein did not increase the liver-succinoxidase activity.

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Toxic Effects of Oxygen at High Pressure on the Metabolism of D-Glucose by Dispersions of Rat Brain

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Previous extensive investigations of the neurochemical toxicity of oxygen at high pressure have resulted in a major problem: the neurotoxic phenomena *in vivo* occurred much more rapidly than did the neurochemical abnormalities *in vitro*. Exposures of men and animals to oxygen at pressures of from 3 to 8 atm. have resulted in the rapid occurrence of such severe neurotoxic phenomena as grand mal convulsions, commonly in less than 30 min. at the higher oxygen pressures (Donald, 1947*a, b*; Dickens, 1955). Experiments *in vitro* required much longer periods of exposure to oxygen at high pressure before toxic manifestations in brain metabolism could be detected. The respiration of brain slices or homogenates was not significantly inhibited for at least 1 hr. during incubations at even the highest of these oxygen pressures (Stadie, Riggs & Haugaard, 1944, 1945*a, b*; Haugaard, 1946, 1955; Dickens, 1946*a, b*, 1955). The review by Dickens (1955) reaffirms this problem and states that 'There is thus a discrepancy between the time course of the two processes [*in vivo* and *in vitro*] which is difficult to explain if poisoning of the brain respiration as a whole is the primary cause of oxygen poisoning'. With the advent of a better understanding of certain neurochemical processes as well as the use of techniques not available to previous workers, we have re-investigated the problem of oxygen toxicity, beginning with the study of cell-free dispersions of rat brain.

Homogenates prepared from whole brain have demonstrated slightly more rapid alterations in the presence of oxygen at high pressure than have brain slices (Stadie *et al.* 1945*a*; Dickens, 1946*a*). Dissolved oxygen is more available to mitochondria, microsomes and other cellular components in dispersions, and the addition of critical enzymes and cofactors can promote and control the optimum utilization of glucose. The observation of a rapid increase in the production of lactate by cell-free dispersions of rat brain at 5 atm. p_{O_2} prompted the measurement of $^{14}CO_2$ production from ^{14}C -labelled substrates during incubation with oxygen at high pressure.

MATERIALS AND METHODS

Chemicals. Uniformly ^{14}C -labelled D-glucose (D-[U- ^{14}C]-glucose), sodium [1- ^{14}C]pyruvate, sodium [2- ^{14}C]pyruvate, sodium [1,4- $^{14}C_2$]succinate, sodium α -oxo[5- ^{14}C]glutarate and sodium DL-[1- ^{14}C]glutamate were obtained from the California Corp. for Biochemical Research (Calbiochem), Los Angeles, Calif., U.S.A. Sodium [1- ^{14}C]acetate and [1- ^{14}C]glycine and additional D-[U- ^{14}C]glucose were purchased from New England Nuclear Corp., Boston, Mass., U.S.A. Additional sodium [1- ^{14}C]pyruvate, sodium [2- ^{14}C]pyruvate and sodium [1- ^{14}C]acetate were obtained from Nichem Inc., Bethesda, Md., U.S.A. Sodium α -oxo[1,2- $^{14}C_2$]glutarate was supplied by Merck, Sharp and Dohme of Canada Ltd., Montreal, Canada.

NAD^+ , NADH, ATP, lactate dehydrogenase, L-glutamate dehydrogenase, o-dianisidine-HCl, hydrazine hydrate