centrations of RNA reflect a differential increase in neural activity associated with the onset of oestrus.

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Mechanism of the Toxic Effects of Hyperbaric Oxygen

The mechanism of the toxic effects of hyperbaric oxygen has long been a subject of discussion and Haugaard[1] has summarized the evidence in favour of the view that the toxicity is due to oxidation of sulphydryl groups. More recently, however, Chance *et al.*[2] observed that 11–17 atm. of oxygen selectively inhibit the energy-linked reduction by succinate of nicotinamide-adenine dinucleotide (NAD). Because this inhibition occurs much more rapidly than oxidation of sulphydryl groups, Chance *et al.* suggested the energy-linked reduction of NAD by succinate as the site of the toxic effects of hyperbaric oxygen. The oxygen pressures used by Chance, however, were much greater than those customarily used therapeutically[3]—that is 3 atm. Because toxicity is also observed at these pressures, it seemed desirable to examine the effects of oxygen at 3 atm. on the energy-linked reduction of NAD by succinate.

In the experiments described here, the reduction of NAD was studied by coupling it to a system which utilizes NADH, namely the synthesis of glutamate from α-oxoglutarate and ammonia[4]. Table 1 shows that oxygen at 3 atm. has no effect on the synthesis of glutamate, even after pre-incubation for 60 min at 25° C. The reaction at 3 atm. of oxygen was carried out in the high-pressure chamber described by Boerema[5].

A sensitive method of following the state of oxido-reduction of the nicotinamide nucleotides in rat liver mitochondria is to measure the deamination of glutamate. Papa *et al.*[8] have shown that deamination is slight when the mitochondrial NADP is kept low by the combined action of the energy-linked reduction of NAD by succinate and the nicotinamide nucleotide transhydrogenase. Inhibition of the former by malonate, for example, causes extensive deamination. The amount of ammonia formed after oxidation of glutamate for 60 min was found to be the same with 3 atm. of oxygen as with 0·2 atm.

Thus according to both tests used, oxygen at 3 atm. was found to have no effect on the energy-linked reduction of NAD by succinate in rat liver mitochondria, and so this system does not appear to be the site of the toxic action of oxygen at this pressure.

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Preparation Parameter for Macromolecules

Some measurable parameter which could characterize the methods of isolation of a natural macromolecule from its source would be useful. Such a parameter, it seems, could be provided by a measure of the polydispersity of the buoyant density of the molecules when in solution and subjected to ultracentrifugation. The parameter of interest is the standard deviation γ of the Gaussian distribution of buoyant densities and can be obtained from centrifugation experiments alone if an empirical relationship between sedimentation coefficient s and molecular weight M is known for the material being investigated. In this communication, the theoretical basis for the use of γ is suggested and some preliminary experimental results for various samples of DNA are given.

If a steady density gradient is created within a liquid held in a cell which is itself subject to centrifugal forces and a macromolecular solute be present, then, provided the solute is either homogeneous or has a Gaussian distribution in buoyant density, the variation of concentration of the solute particles with distance down the cell will follow a Gaussian distribution. According to Sueoka[1], the density ρ varies with the distance r from the axis of rotation according to the expression

$$\rho = \rho_0 + A\,(r^2 - r_0^2) \qquad (1)$$

where ρ_0 is the mean density at distance r_0 and A is a constant for the particular gradient.

According to Meselson *et al.*[2], if σ is the standard deviation of the concentration versus distance curve, the spread of which results from thermal agitation alone, then for a sample homogeneous in both molecular weight and density,

$$M = RT\,\{\bar{v}\,\omega^2\,r_0\,\sigma^2\,(\mathrm{d}\rho/\mathrm{d}r)_{r=r_0}\}^{-1} \qquad (2)$$

in which $\bar{v}$ is the apparent partial specific volume of the macromolecules, ω is the angular velocity and the other symbols have their usual meanings. If, however, the isolation procedure is such as to produce material which

Table 1. EFFECT OF 3 ATM. OF OXYGEN ON THE SYNTHESIS OF GLUTAMATE BY RAT LIVER MITOCHONDRIA COUPLED WITH THE AEROBIC OXIDATION OF SUCCINATE

Series:	1		2		3	
No. of experiments:	11		6		5	
	0·2 atm. O_2	3 atm. O_2	0·2 atm. O_2	3 atm. O_2	0·2 atm. O_2	3 atm. O_2
ΔO (μatoms/mg)	3·08 ± 0·20	3·77 ± 0·28	4·46 ± 0·25	5·56 ± 0·30	3·54 ± 0·76	5·00 ± 1·25
Δ Glutamate (μmoles/mg)	1·17 ± 0·10	1·17 ± 0·09	2·57 ± 0·45	2·50 ± 0·45	0·95 ± 0·23	0·97 ± 0·20

Rat liver mitochondria, isolated by the method of Myers and Slater[6], were suspended in a medium containing 15 mmolar KCl, 2 mmolar EDTA, 25 mmolar *tris*-HCl buffer (*p*H 7·4), 10–15 mmolar potassium phosphate buffer (*p*H 7·1), 0·1 mmolar ADP, 25 mmolar sucrose (derived from the mitochondrial suspension), 24 mmolar α-oxoglutarate, 24 mmolar NH_4Cl, 1 mmolar arsenite, 25 mmolar succinate, and, in series 2, 10 mmolar ATP. The concentration of mitochondria varied between 3 and 6 mg protein/ml. In series 3, the suspension was pre-incubated for 1 h at 25° C before addition of succinate. Reaction at 25° C for 60 min. Uptake of oxygen was measured in Warburg flasks with 0·1 ml. 30 per cent (w/v) potassium hydroxide in the centre well. The reaction was stopped with 0·3 ml. 35 per cent (w/v) perchloric acid, and glutamate determined according to Bernt and Bergmeyer[7]. The values given are means ± standard error.