Decrease of Phosphorus/Oxygen Ratios by Dinitrophenol *in vivo*

In a paper by one of us[1], it was reported that intraperitoneal administration of 2 : 4-dinitrophenol produces a high degree of uncoupling of oxidative phosphorylation in mitochondria from several rat tissues. There were histological changes in these tissues, which showed mitochondrial swelling and cloudy swelling. In the case of the liver, we have found that very shortly after treatment, fat droplets appear in the cells, followed later by glycogen. Similar changes were observed by Fonnesu and Severi[2,3], who found also that cloudy swelling is characterized by decrease of phosphorus/oxygen ratios. In addition, treated rats showed increase of body temperature, a condition which has been referred by many authors to the decline of phosphorylation[4,5].

In a recent communication in *Nature*, Parker[6] could not confirm the inhibition by 2 : 4-dinitrophenol of oxidative phosphorylation *in vivo*. His work, however, differs from ours in two important ways: (1) the nature of the substrate used, which was α-ketoglutarate in our experiments, glutamate in those of Parker; (2) the amount of mitochondria used for each determination, which corresponded to 100 mgm. liver in our experiments, and to 250 mgm. in Parker's. With regard to point (1), Saktor and Cochran[7] have found that the phosphorylation coupled with the oxidation of ketoglutarate by housefly muscle mitochondria is more sensitive to 2 : 4-dinitrophenol than that coupled with the oxidation of glutamate. With regard to point (2), one may observe that the mitochondrial membrane is the limiting factor in many enzymic reactions, and that the use of too large amounts of mitochondria may then be the cause of unexpected results.

In order to check the influence of these differences in the methods, the experiments have been repeated, using different amounts of mitochondria (corresponding to 100 and 250 mgm. of wet liver, respectively), and two substrates (ketoglutarate and glutamate). Since Hunter and Ford[8] have shown that loss of oxidative phosphorylation occurring after mitochondrial swelling *in vitro* can be partially restored by addition of diphosphopyridine nucleotide, 1×10^{-3} *M* diphosphopyridine nucleotide was added in each Warburg flask. The suspension medium for the preparation of the homogenate was 0·25 *M* sucrose containing 0·002 *M tris* buffer, *p*H 7·6. The other experimental conditions were those described in the previous paper[1].

Table 1. Uncoupling of Oxidative Phosphorylation in Rat Liver Mitochondria after Treatment with 2 : 4-Dinitrophenol (3 mgm./100 gm. Body-weight). Eight Experiments for Each Group. Standard Deviation is given for Each Average

Amount of mitochondria (mgm. liver)	Substrate	Treatment	μ Atoms P	μ Atoms O	P/O
250	α-Keto-glutarate	None	14·67±2·8	4·33±0·8	3·39±0·2
100	α-Keto-glutarate	None	11·52±1·6	3·37±0·4	3·41±0·2
250	Glutamate	None	11·76±2·7	4·19±0·9	2·80±0·2
100	Glutamate	None	8·47±1·9	3·29±0·7	2·57±0·3
250	α-Keto-glutarate	DNP	7·29±2·3	3·05±0·8	2·39±0·5
100	α-Keto-glutarate	DNP	2·28±1·8	1·67±0·7	1·36±0·6
250	Glutamate	DNP	6·78±1·4	3·14±0·4	2·16±0·3
100	Glutamate	DNP	3·96±2·1	2·53±0·9	1·56±0·4

It is clear from Table 1 that a decrease of phosphorus/oxygen ratios was observed again in rats receiving 3 mgm. 2 : 4-dinitrophenol/100 gm. body-weight. The extent of decrease, which was statistically significant for each treated group, was, however, higher with ketoglutarate than with glutamate, and with mitochondria corresponding to 100 mgm. liver. The values obtained with mitochondria from 250 mgm. tissue were never 2·5 times as great as those obtained with the particles from 100 mgm. tissue. Oxidation of both substrates was decreased after treatment with 2 : 4-dinitrophenol, but the decrease of phosphorylation was always higher. Both differences in the methods may, therefore, have co-operated in the production of the discrepancy between the results obtained by Parker and our results.

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The Antihæmophilic and Christmas Factor Activities of Ethanol Fractions of Brain Extract

The activity of brain tissue extract in the mechanism of blood coagulation is considered to be equivalent to a combination of platelets, antihæmophilic globulin and Christmas factor[1] (plasma thromboplastin component). The latter two blood-clotting factors have been separated in different ethanol fractions of plasma. It was thus of interest, following our report on the isolation of antihæmophilic globulin from brain tissue[2], to study the antihæmophilic and Christmas factor activities of various ethanol fractions obtained from brain extract.

Heparinized human brain extract was prepared as previously described[2]. The activity of each fraction was determined by its effect on the blood and plasma-clotting time, prothrombin consumption and thromboplastin generation test.

On treating the brain extract in a way similar to that described by Cohn *et al.*[3] in 1946, for plasma fractionation, traces of these two factors were present in the products corresponding to fractions I, II+III, IV−1 and IV−4. However, using the modified method of Cohn *et al.*[4], the two factors were present in the precipitate obtained at 0·04 *M* sodium concentration, 0·066 *M* fraction ethanol and *p*H 5·8 (corresponding to fraction I+II+III of plasma[4]). Paper electrophoresis of this precipitate using barbitone buffer, *p*H 8·6, showed two bands: one at the origin and another occupying the β-globulin position. This fraction was found to contain no heparin. The latter was precipitated with most of the remaining proteins on the addition of 0·02 *M* zinc acetate.

The following experiments were carried out with the precipitate containing the Christmas factor and antihæmophilic globulin in an endeavour to obtain each factor separately. A reagent[4] containing 0·6 *M*