change in the size of the glomerulosa.

Summary. Adult rats fed the drug DDD rapidly develop signs of some adrenal dysfunction; decreased eosinophil response, decreased uric acid/creatinine ratio, increased insulin sensitivity and decreased response to stress of cold. The urinary and plasma Na and K are not affected.

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Localization of Glycolytic and Respiratory Enzyme Systems on Isolated Mouse Brain Mitochondria.* (20266)

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The Krebs cycle has been demonstrated using cyclophorase preparations, *i.e.*, suspensions of washed homogenate pellets(1). On the other hand, the investigators who have worked with isolated mitochondria prior to this time have demonstrated many of the Krebs cycle reactions, but never the malonate-sensitive oxidation of pyruvate denoting the complete cycle(2-9). These findings are in accordance with the fact that isolated mitochondria have not previously been reported to show the reversible staining with Janus green B which is characteristic of mitochondria *in vivo*(10). The absence of this special staining reaction indicates that the functional intactness of these mitochondria was apparently of too low a degree for them to carry the complete Krebs cycle. Glucolysis has been studied in extracts of various tissues(11-15). This process has, however, never before been demonstrated for isolated cell constituents used singly or in combination. Glycolysis, with hexose-diphosphate in the presence or absence of glucose as substrate, has been reported to occur in the supernatant(16-18); supernatant plus submicroscopic particles(6); or in the supernatant, in the submicroscopic particles, and in the mitochondria(19). No report on the Janus green B staining reactions of these preparations was given. The present paper briefly presents data which demonstrate that both these enzymic sequences occur in balanced quantities on mouse brain mitochondria isolated and washed in 0.25 M sucrose.

Materials and methods. Webster strain mice were killed by cervical dislocation and the entire brain immediately removed. The tissue was collected in ice-cooled 0.25 M sucrose solution at pH 7.4-7.7. Razor-cut slices were made from brains in a small amount of fresh sucrose medium in a petri dish set in ice. Slices were drained of solution, weighed and placed directly in manometric vessels. Brain tissue used to prepare mitochondria and supernatant was homogenized in a pyrex glass test tube fitted with a plastic plunger, and suspended in 9 volumes of fresh sucrose medium. It was then subjected to one 45-60 second clearance run at up to 7000 g, in a refrigerated centrifuge, to remove nuclei, whole cells and cell fragments.

* These data are from a thesis to be presented to the Faculty of the Graduate School of Georgetown University by M. L. Hesselbach in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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The volume of the remaining suspension was measured and it was then centrifuged at 25,000 g for 20 minutes to yield a first supernatant and a mitochondrial pellet. The mitochondria were washed by resuspension to their original volume with sucrose medium and centrifugation for 60 minutes at 25,000 g. The particulates were ready for use when this pellet had again been resuspended to its original volume in sucrose solution. The first supernatant was also centrifuged for 60 minutes at 25,000 g to clear it of the remaining tissue particles. The relative purity of the mitochondrial preparations‡ which carried out the described activities must be emphasized. The fractions were checked microscopically. Most of the cellular debris was carefully removed in the clearance run. The use of a non-ionic medium throughout the preparative steps minimized the possible aggregation of submicroscopic particles to a point where they would sediment in the mitochondrial pellet. It also minimized the direct association of these particles with the mitochondria. Furthermore, the mitochondria were washed in order to remove adherent material. The large mitochondrial pellet was cream-colored and opaque as is characteristic of these particulates. The long, high speed centrifugation given the supernatant to remove tissue contaminants, yielded, in contrast to the above, a small, semi-transparent, cream-colored gelatinous pellet, typical of the submicroscopic particles present in these preparations. Tissue was ice-cooled throughout all manipulations until the Warburg vessels were placed on manometers. Gassing was done with manometers in place on a 38°C bath. About 20 minutes elapsed between the time the vessels were taken out of the ice-bath until readings were begun. Gas exchange under aerobic (100% O_2) and anaerobic (95% N_2/5% CO_2) conditions was measured by the usual manometric technic. The coenzyme and other chemicals used in these experiments were obtained commercially. Nitrogen content was measured by the Kjeldahl technic.§ The lactic acid which accumulated both anaerobically and aerobically was measured chemically by the Barker-Summerson method§(20). (Anaerobic lactic acid formation was also measured manometrically, as these vessels contained bicarbonate.)

Results and discussion. Glucose was used as substrate in all experiments reported here. The results are summarized in Table I. It can be seen that only the preparations which contained mitochondria showed oxygen consumption in the presence of glucose. That these particulates, even when isolated and washed, carried the complete glycolytic system was shown by the accumulation of lactic acid under both aerobic and anaerobic conditions. That they carried, in addition, the complete Krebs cycle was demonstrated by their sustained activity under aerobic conditions, and by other experiments which proved that oxygen consumption under these conditions was malonate-sensitive.

In contrast, the supernatant did not show any oxygen consumption with glucose as substrate. It did, however, produce lactic acid aerobically and anaerobically.

Results of the lactic acid determinations are presented both on a whole-tissue-equivalent basis (total lactic acid formed per vessel) and on a nitrogen basis; so that the two sets of relationships may be observed.

Mention should be made of the submicroscopic particle pellet described in the materials and methods section. When it was tested for glycolytic and respiratory activity with the same cofactors as supplied to the other fractions, neither enzyme system could be demonstrated. Similar fractions have been found by other workers to carry various single enzymes and component chains from both systems. Since their enzyme content varies with method of preparation, it is improbable that they occur preformed in the intact cell.

It was noteworthy that a quantitative rela-

‡ Proof of the good condition of the isolated mitochondria was that they stained and destained with 0.01% Janus green B. (Unpublished work of Showacre and duBuy, this laboratory.)

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TABLE I. Aerobic and Anaerobic Glucose Utilization by Mouse Brain Preparations.

Fraction, duration	Substrate	Gas phase: 100% O_2 μl O_2 /mg N	100% O_2 γ lactic /vessel	100% O_2 γ lactic /mg N	100% O_2 Final pH	Gas phase: 95% N_2/5% CO_2 μl CO_2 /mg N	95% N_2/5% CO_2 γ lactic /vessel	95% N_2/5% CO_2 γ lactic /mg N	95% N_2/5% CO_2 Final pH
Slices, 120 min.	0	129, 96	38*	41*	7.24	45, 40	124	135	7.82
	Glucose	223, 230	1548	1681	6.53	208, 201	1464	1593	7.26
Mitochondria, 120 min.	0	78, 84	70*	103*	7.41	65, 59	63*	92*	7.90
	Glucose	274, 273	332	487	7.20	198, 200	742	1090	7.59
Supernatant, 120 min.	0	10, 9	58	145	7.22	99, 95	89, 85	223, 213	7.90
	Glucose	7, 5	1616	4050	6.78	326, 352	1260, 1140	3158, 2860	7.51
Mitochondria and supernatant, 90 min.	0	106, 91	71*	56*	7.24	11, 12	129	101	7.79
	Glucose	179, 226	1320	1031	6.53	129, 122	1232	965	7.21

* These preparations did not show the characteristic purple in the lactic acid test, but only the slight green interference color given by fructose.

The main compartment of each vessel contained: 8.7 mg K_2HPO_4, 0.43 mg diphosphopyridine nucleotide, 5.1 mg niacinamide, 0.85 mg adenosine triphosphate and 0.825 mg $MgSO_4$. The anaerobic vessels contained, in addition, 4.0 mg $KHCO_3$. The center well of each aerobic vessel contained 0.1 ml 2N KOH. 40 mg wet wt of slices in the indicated vessels. Concentration of fractions in each vessel was comparable to the calculated concentration of these fractions in the vessels containing 40 mg of slices. 6.7 mg anhydrous glucose was added last, directly to main compartment of indicated vessels. Tissue and additions were prepared in 8.6% sucrose to give a final volume of 1 ml per vessel. Gas values are based on individual vessels. Lactic acid and pH determined on the contents of a number of the same vessels.

tionship existed between glycolysis and respiration on the mitochondria. Under aerobic conditions, only the particulates prevented the accumulation of lactic acid to the extent that they respired (2.5 μ M O_2 equivalent to 1 μ M pyruvate). This relatively good balance between glycolysis and respiration allowed mitochondrial metabolism of glucose to continue for as much as 6 hours, in the presence of air or oxygen. The other fractions in contrast, formed as much lactic acid aerobically as anaerobically. (See columns 5 and 10 of Table I for pH values.)

It is reasonable to assume that some of the Krebs cycle components had washed off, as diphosphopyridine nucleotide and adenosine diphosphate had to be supplied for maximum activity. However, the enzymes of the cycle must have remained bound to the mitochondria to a great degree, since neither glucose nor pyruvate, sparked with traces of succinate, nor succinate itself, in substrate amounts, was respired by the supernatant. This conclusion is further supported by the fact that the respiratory activity of the isolated mitochondria approached that of the slices. The activity of the Krebs cycle was here dependent on an adequate supply of substrate from the glycolytic system. Since the high percentage of Krebs cycle activity retained on the isolated mitochondria was maintained by their glycolytic system, it follows that a major part of this system was also bound on the washed particulates.

The balance between the glycolytic and Krebs cycle activities may very well be dependent on the orientation of the components of these systems on the mitochondrial structure. It is recognized that some changes occur in the mitochondria during isolation. These changes might result in sufficient disorientation of glycolysis to show the rather small excess of glycolytic activity over Krebs cycle activity of the washed mitochondria. The glycolysis carried out by the supernatant may then represent the activity of only a small fraction of the total system originally on the mitochondria, now no longer limited by the coordinating effect of the particulate structure. The particulate-bound glycolytic and respiratory systems might function like two endless belt systems, linked by pyruvate.

Summary. 1. Washed mouse brain mitochondria carried out the complete enzymic conversion of exogenous glucose to carbon dioxide and water. Glucolysis took place anaerobically and aerobically, with the production of lactic acid. Under aerobic conditions glucose was oxidized by means of a malonate-sensitive cycle. The accumulation of lactic

acid was correspondingly decreased. 2. In contrast, the supernatant carried out anaerobic and aerobic glucolysis, but not respiration of glucose. The submicroscopic particle fraction did not carry out the reactions of either system.

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Nature of Growth Inhibitor in Some Mammalian Sera For Pleuropneumonia-Like Organisms of Human Origin. (20267)

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The inhibitory effect of some mammalian sera, when used in a concentration greater than 10%, on the growth of pleuropneumonia-like organisms (PPLO) has been noted by several investigators. Rabbit, sheep, and horse sera are satisfactory supplements but guinea pig, steer, and cow sera are inferior for the cultivation of the bovine pleuropneumonia organism(1). Growth of this organism was partially inhibited by 20% and completely inhibited by 50% horse serum. Edward(2), likewise, found that 17 strains of pleuropneumonia organisms of various origins grew poorly when bovine serum was used as a supplement. Morton, Smith and Leberman (3) compared various mammalian sera as supplements for the cultivation of human strains of PPLO. Bovine serum and citrated human plasma added to the basal medium in concentrations exceeding 10% were definitely inhibitory. Separation of the active growth factor in bovine serum eliminated this growth inhibitor(4). Since the fractionation of serum to obtain the growth factor removed the globulins, it was speculated that the inhibitor might be associated with antibody against PPLO. This report shows that the inhibitory component of bovine plasma is associated with alpha-globulin and the inhibitory action is enhanced by the presence of complement.

Materials and methods. The method employed for measuring the inhibitory activity of various fractions was essentially the same as previously reported(4). The preparations to be tested were incorporated into Bacto-PPLO agar with added 1% fraction A derived from bovine serum. Fraction A is the serum fraction containing the protein growth factor required by some strains of PPLO of human origin and has been described previously(4).

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