Fluorescence must be in the cytoplasm or nucleus of individual cells to be a valid indicator of HC viral antigen.

Enhancing factor(s) in the guinea pig complement aided union of A-AB to give a visible FA reaction. A similar requirement was indicated by Liu *et al.*(15) for a FA test for primary atypical pneumonia.

This preliminary report is presented primarily because the U. S. Dept. of Agriculture is embarking on an HC eradication program. Present methods for specific identification of HC involve inoculation of groups of susceptible and immune swine. The method is expensive, and tests take up to 10 days for tentative evaluation. The FA method reported by Dunne(16) is indirect since it involves inoculation of TC with HC virus several days before the FA test. The FA method herein described takes less than 2 hours to accomplish the same purpose. Obvious refinements need to be made to provide diagnostic procedures adequate for routine use.

Summary. A hog cholera virus-fluorescent antibody system has been developed using pathogen-free pigs as the source of globulins. Heterologous globulin was developed by hyperimmunizing pathogen-free genetically small pigs with 3 strains of virulent hog cholera virus. Success of the system appeared to be based on use of pigs having minimum of non-specific globulins, complement for antigen-antibody union, and globulins which approach maximum levels of conjugation to fluorescein isothiocyanate. Use of this system in the study of viral infections of swine embryos and for rapid diagnosis of hog cholera are discussed.

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Influence of Alpha-Ketoacids on the Respiration of Brain *in vitro*.* (28456)

ROY K. HOWELL and MELVIN LEE
(Introduced by Harold A. Harper)
Department of Preventive Medicine, University of California School of Medicine, San Francisco

Several inherited defects in amino acid metabolism are characterized by mental retardation and central nervous system dysfunction, as well as by elevated plasma and urine concentrations of particular amino acids or amino acid metabolites(1). These include phenylpyruvic oligophrenia(2), citrullinuria(3), hyperprolinemia(4), hydroxypro-

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linemia(5), Maple Syrup Urine Disease(6), and leucine sensitivity(7). Maple Syrup Urine Disease (Branched Chain Ketoaciduria) appears to be related to an inability to metabolize the alpha-ketoacid analogs of leucine, isoleucine, and valine(8), while leucine sensitivity is related to the metabolism of alpha-ketoisocaproic acid, the alpha-ketoacid derived from leucine(7).

In an attempt to establish a possible relationship between the defect in amino acid metabolism and the central nervous system abnormalities common to these diseases, we have investigated the effects of several amino acids and their alpha-ketoacid analogs on the respiration of rat brain homogenates and rat brain slices.

Experimental. Male Long-Evans rats, weighing 150-200 g, and maintained on a commercial rat diet, were used for all experiments. Animals were sacrificed by cervical fracture followed by decapitation. The entire brain was rapidly removed, intact, and immersed in iced Krebs-Ringer-phosphate buffer, pH 7.4.

Homogenate experiments. The entire brain was homogenized for 1-2 minutes in Krebs-Ringer-phosphate buffer, pH 7.4, to make a concentration of 100 mg tissue per ml of buffer. An all-glass Potter-type homogenizer was used(9). One ml of the appropriate substrate, in Krebs-Ringer-phosphate buffer, was placed in the outer chamber of each 15 ml Warburg vessel and 0.2 ml of 10% potassium hydroxide was placed in the center well. One ml of homogenate was then added to the outer chamber. The flasks were attached to the manometers and equilibrated at 37°C for 5 minutes. Manometer readings were taken at 15 minute intervals for 120 minutes. Control flasks, in which the amino acid or alpha-keto-acid substrate was omitted, were prepared with each experiment. A thermobarometer was included in each experiment. All determinations were carried out in triplicate.

Brain slice experiments. To obtain an equal distribution of white and gray matter in each flask, hemispheres of each brain were trisected. The trisections were cut, using a McIlwain Tissue Chopper, into slices 0.35 mm thick. Slices from each trisection were brought together and approximately 100 mg of slices, in 1 ml of Krebs-Ringer-phosphate buffer, were placed in the outer chamber of the Warburg Flask. Otherwise, the slice experiments were carried out as described for homogenates.

TABLE I. Effect of Amino Acids and Alpha-Ketoacids on Oxygen Uptake by Rat Brain Homogenates.

Substrate	No. exp	Oxygen uptake* 60′	120′
Controls	11	.52 ±.03	.98 ±.06
L-leucine	5	.49 ±.01	.96 ±.03
Alpha-ketoisocaproic acid	3	.47 ±.02	.94 ±.05
L-valine	4	.49 ±.03	.93 ±.03
Alpha-ketoisovaleric acid	3	.50 ±.04	.95 ±.06
L-phenylalanine	1	.50	.93
Phenylpyruvic acid	1	.53	1.02
Phenylacetic "	1	.51	.99

* Change in manometer reading per mg of tissue. Means and standard deviations given. All substrates .01 M.

Substrates. *L*-valine (cfp grade) and phenylpyruvic acid (A grade) were obtained from California Corporation for Biochemical Research. *L*-leucine, *L*-phenylalanine, alpha-ketoisovaleric acid, alpha-ketoisocaproic acid, and phenylacetic acid were obtained from K and K Laboratories, New York. The purity of the alpha-ketoacids was established by the paper chromatographic method of Meister and Abendschein(10), using 1-butanol-water-ethanol. The pH of the substrates in buffer was readjusted to pH 7.4 if necessary.

In the slice experiments, flasks were removed at 60 and 120 minutes and the pH of the reaction mixture was determined, using a Beckman Zeromatic pH meter.

All values are reported as changes in manometer reading per mg of tissue in the time specified and are averages of 3 flasks.

Results and discussion. Table I shows results of homogenate experiments. At concentrations of 0.01 M, *L*-leucine, *L*-valine, alpha-ketoisocaproic acid and alpha-ketoisovaleric acid do not depress oxygen uptake to any statistically significant extent. Only one experiment was carried out with *L*-phenylalanine, phenylpyruvic acid, and phenylacetic acid, but no evidence of an inhibitory action by any of these substrates was noted.

TABLE II. Effect of Amino Acids and Alpha-Ketoacids on Oxygen Uptake by Rat Brain Slices.

Substrate		Oxygen uptake* Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	%
Control	60′	.93	1.32	1.47	1.64	1.47	1.60	1.77	100
	120′	1.96	2.70	2.88	3.15	2.92	2.75	3.28	100
L-leucine	60′	.96	1.28	—	—	—	—	—	100
	120′	1.98	2.60	—	—	—	—	—	98.5
L-valine	60′	—	1.30	—	—	—	—	—	98
	120′	—	2.58	—	—	—	—	—	96
L-phenylalanine	60′	—	—	1.42	1.52	—	—	—	95
	120′	—	—	2.76	2.96	—	—	—	95
Alpha-ketoisocaproic	60′	.90	1.20	1.24	—	1.33	1.44	—	90
	120′	1.62	2.36	2.30	—	2.60	2.75	—	85
Ketoisovaleric	60′	—	1.10	1.10	—	1.25	1.37	—	82
	120′	—	2.00	1.94	—	2.31	2.50	—	75
Phenylpyruvic	60′	—	—	1.22	1.10	—	—	1.28	83
	120′	—	—	2.36	2.10	—	—	2.31	80
Phenylacetic	60′	—	—	—	1.32	—	—	1.53	83
	120′	—	—	—	2.50	—	—	2.68	84

* Change in manometer reading per mg of tissue. Figures are for individual experiments.

Table II shows the results of brain slice experiments. Since the rate of oxygen utilization differed from one experiment to the next, the data for individual experiments are shown. In the last column of the Table, the mean oxygen uptake with each substrate is expressed as a percentage of that of the control. *L*-leucine, *L*-valine, and *L*-phenylalanine had no significant effect on oxygen uptake. However, after 120 minutes alpha-ketoisocaproic acid had depressed oxygen uptake by 15% and alpha-ketoisovaleric acid depressed it by 25%. The deamination product of phenylalanine, (phenylpyruvic acid), and phenylacetic acid depressed oxygen utilization to a marked extent (20% and 16%, respectively), after 120 minutes.

It was felt that these inhibitory activities might be artifacts due to acidification of the medium by some of the substrates. For this reason, the pH of the incubation medium was measured after 60 and 120 minutes of incubation. This was done only in Experiments 5, 6, and 7. The pH of the control flasks was 6.5 to 6.9 and that of the flasks with substrates was always within 0.1 pH unit of the controls for that particular experiment.

Finally, it should be noted that in all experiments, the oxygen uptake of brain slices (per mg tissue) was approximately 3 times as great as that of homogenates. This decrease in oxygen consumption by the homogenates is presumably due to the disruption of cellular organization. The inhibitory action of alpha-ketoacids on respiration by brain slices and the failure to demonstrate inhibition when homogenates were employed suggest that the alpha-ketoacids may be acting at the cell membrane, possibly by affecting a transport mechanism. However, the data do not rule out the possibility of an action on some intracellular structural unit as well.

These data are consistent with the observation of Patrick(11) that in brain tissue from patients with Maple Syrup Urine Disease transamination is normal, whereas oxidative decarboxylation is impaired. In such circumstances, ketoacids might be expected to accumulate in the tissue.

It may seem paradoxical that the ketoacids were found to exert an inhibitory effect on respiration of brain slices when the parent amino acids from which the ketoacids are derived did not do so. The explanation may lie in the possibility that the amino acids are either not deaminated at all in the extracellular compartment or are deaminated at such a slow rate that inhibitory levels of ketoacids did not develop.

Summary. 1. The effects of *L*-leucine, *L*-valine, *L*-phenylalanine, alpha-ketoisocaproic acid, alpha-ketoisovaleric acid, phenylpyruvic

acid, and phenylacetic acid on oxygen consumption by slices or homogenates of rat brain were measured. 2. None of these substrates significantly inhibited oxygen uptake by rat brain homogenates. 3. *L*-leucine, *L*-valine, and *L*-phenylalanine were also found not to significantly depress oxygen uptake by brain slices. 4. On the other hand, alpha-ketoisocaproic acid, alpha-ketoisovaleric acid, phenylpyruvic acid, and phenylacetic acid (at concentrations of 0.01 M) did depress oxygen utilization by brain slices to the extent of 10-27% of control values. 5. It is suggested that the central nervous system dysfunction observed in patients with certain hereditary diseases characterized by defects in amino acid metabolism may be related to the accumulation of alpha-ketoacids which occurs in these diseases.

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Biological Activities of the 2-Acetofuran Derivative of 16α, 17α-Dihydroxyprogesterone. (28457)

LEONARD J. LERNER, EURIPIDES YIACAS and ALECK BORMAN
Squibb Institute for Medical Research, New Brunswick, N. J.

A new class of progestational steroids derived from 16α, 17α-dihydroxyprogesterone was reported by Fried *et al.*(1). One of these compounds, the acetophenone derivative of 16α, 17α-dihydroxyprogesterone (D)* was shown to be more active as a progestational agent in the rabbit than progesterone (P) parenterally administered or 17α-ethinyl-19-nortestosterone (N)† orally administered(2). This progestogen maintained pregnancy in the ovariectomized rat(3), did not masculinize the rat fetus(4) and was devoid of undesirable hormonal activities(2).

Another member of this series of steroids is the 2-acetofuran derivative of 16α, 17α-dihydroxyprogesterone (AF). The synthesis of this compound was described by Fried *et al.*(5). Results of biological studies with this compound are presented here.

Materials and methods. New Zealand white rabbits weighing 800-1200 g were employed in the progestational evaluation assays. The animals were primed with a total of 15 μg estradiol benzoate, administered subcutaneously in 3 divided doses on alternate days for a 6-day period. The progestational steroids were administered in a sesame oil solution or suspension, subcutaneously or orally once daily for the next 5 days. Animals were sacrificed 24 hours after the final dose. The uteri were removed and weighed, and sections were taken for histological evaluation(6).

Duration of activity was determined in ovariectomized immature rabbits. These animals were primed with estrogen as described above. A single 5 or 10 mg dose of progestogen was administered intramuscularly on the day following the last priming dose. A main-

* Droxone or Deladroxone (Dihydroxyprogesterone Acetophenide—Squibb®).

† Norethisterone.