ens were selected for a parallel study of both viruses because they are not known to be susceptible to natural infection by either. In contrast, dogs injected experimentally with MV would be constantly under the threat of a natural CD infection, sometimes even under the most careful isolation conditions. Extension of this study to monkeys and ferrets is in progress, as is the testing of sera from humans who received injections of either an experimental measles vaccine or the chick-embryo-adapted strain of avirulent CD virus.

Summary. Puppies, vaccinated against canine distemper (CD) or immunized and challenged with CDV, developed high levels of homologous antibodies but failed to develop CF or serum neutralizing antibodies for measles virus (MV). Similarly, chickens hyperimmunized with CD virus did not develop measles neutralizing antibodies, although CD antibodies in appreciable levels appeared in 6 of 6 birds. Hyperimmunization of another group of chickens with MV was followed by no neutralizing CD antibodies in any of 6, whereas 5 of 6 birds gave a substantial measles response.

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Effects of Amines and Monoamine Oxidase Inhibitors on Hypothalamic Succinic Dehydrogenase Activity.* (24893)

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Although the mammalian hypothalamus appears to modulate certain activities of the anterior lobe of the hypophysis, the mechanisms remain obscure. Contributions by amines to stimulation of parts of the hypothalamus and perhaps hypothalamo-hypophyseal transmitter systems appear likely, due to: (A) the especially high concentration of certain of these amines as well as monoamine oxidase in the hypothalamus(1,2); (B) pharmacologic evidence of a close connection between central sympathetic stimulation and turnover of diencephalic and mesencephalic sympathin (3); and (C) elevation of posterior hypothalamic electrical activity, respiration and aerobic glycolysis following epinephrine administration(4,5) and elevation of supraoptic and paraventricular acetylcholinesterase and acid phosphatase after administration of serotonin(6). The work reported here employs succinic dehydrogenase system as an anaerobic test system for evaluating effects of physiologically significant amines, related compounds and monoamine oxidase inhibitors. Succinic dehydrogenase activity increases during functional stimulation in several glandular and protein-synthesizing tissues. The present work aims to determine whether this enzyme activity in the hypothalamus responds to amines and drugs possibly active in

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regulating or modifying hypothalamic function *in vivo*.

Methods and materials. Adult rats (Long-Evans strain), golden hamsters (*Mesocricetus auratus,* Waterhouse), and deer mice (*Peromyscus maniculatus,* Wagner) born and raised in our laboratory were used. The deer mice are from 2 inbred strains derived from feral individuals taken in Washtenaw County, Mich. (= W-M) and San Benito County, Calif. (= SB-C). Within each experiment, littermates were evenly distributed, and in rats the age range was less than 2 weeks. During each experiment animals were individually caged and maintained at 22-26°C with 14 hours of light/day; food and water were available *ad lib*. All injections were intraperitoneal and all experiments contained control groups injected with the solvent, water or 0.85% sodium chloride. Untouched controls were included as well in some experiments. Succinic dehydrogenase activity was measured by reduction of 2, 3, 5-triphenyl-2H-tetrazolium chloride (Eastman) by the enzyme system and colorimetric determination of the resulting formazan. This method is derived from that of Seligman and Rutenberg(7) and subsequent authors. The anterior and posterior halves of the hypothalami were immediately dissected from rapidly decapitated animals and were cut into small pieces while in a drop of 0.85% sodium chloride. Pieces from each area were rinsed for exactly 15 minutes at 24-26°C in 4 ml pH 8.25 phosphate buffer + sodium cyanide (1:2 dilution of solution 1 below) to remove endogenous substrates, and then incubated at 37°C with mild agitation for 2 hours in 1.5 ml of freshly prepared medium containing equal parts of the following 3 stock solutions and adjusted to pH 8.25: (1) 0.225 M sodium phosphate buffer, 0.1% sodium cyanide; (2) 5.4% sodium succinate, .01 M $AlCl_3$, .001 M $CaCl_2$; and (3) 0.4% tetrazolium. Incubations in control media (lacking succinate) failed to produce significant amounts of formazan in the tissue and minor variations in size of tissue fragments were without significant effect. After incubation, tissues were rinsed in distilled water, fixed in 10% neutral buffered formalin, dried overnight at 37°C on previously weighed aluminum foil pans, weighed to nearest μg with quartz helix balance (Microchemical Specialties Co., Berkeley), and extracted with ethyl acetate and grinding. The extracted formazan was rapidly measured at 490 mμ and the μ moles of tetrazolium reduced/μg of dry tissue were computed by method similar to that used by Shelton and Rice(8). The 2 sides of the anterior and posterior hypothalami of rats and hamsters were determined separately and later averaged before the average of each experimental group was computed.

Results. Monoamine oxidase inhibitors: 1-isonicotinyl 2-isopropyl phosphate (Marsilid or Iproniazid) at 170 mg/kg body weight (20 ♀ rats, 15-16 hr) and at 30 mg/kg (20 ♂ rats, 20-21 hr) produced no change in hypothalamic succinic dehydrogenase activity (HSD). Likewise in deer mice (W-M ♀) this drug was ineffective both alone (200 mg/kg, 18-21 hr) and when followed by 5-hydroxytryptophan (15 mg/kg, 5-7 hr), which may pass the blood-brain barrier and raise the brain level of serotonin under these conditions(2). The more potent and longer-acting inhibitor, beta-phenylisopropylhydrazine hydrochloride (JB-516 or PIH)(9) significantly depressed HSD in both rats and deer mice (Table I). In hamsters doses of 30-50 mg/kg produced severe behavioral agitation, but significant change in HSD did not occur, even when 5-hydroxytryptophan was injected 5-6 hr prior to autopsy. The sympathetomimetic amine, amphetamine, which differs from PIH in having an amino in place of the hydrazino group and which has been considered an inhibitor of amine oxidation (10), failed to modify HSD in ♂ rats (N = 47, 25-30 mg/kg) either 18-20 or 5-6 hours later. Reduction of HSD by PIH (20-30 mg/kg) in rats was not significantly modified when followed 5-8 hours before autopsy by 5-hydroxytryptophan (18 mg/kg), 1-3, 4-dihydroxyphenylalanine (7 mg/kg), 1-epinephrine bitartrate (2.5 mg/kg), or 1-norepinephrine (1.5-2.0 mg/kg). Similarly, in deer mice (SB-C) dl-5-hydroxytryptophan • H_2O, 1-3-4-dihydroxyphenylalanine, or both together (each 16 mg/kg) failed to modify the response to PIH.

TABLE I. Reduction of Hypothalamic Succinic Dehydrogenase after Administration of PIH.

Species	Sex	No. of animals	Time, hr†	Dose, mg/kg body wt	AH	P	PH	P
Rats	♀	23	18–20	0	93.69 ± 2.69*		93.79 ± 2.29*	
		16	"	30	85.82 ± 1.75	<.05	82.33 ± 2.57	<.01
"	"	10	19–20	0	86.76 ± 1.44		82.95 ± 3.93	
		6	"	90	57.60 ± 4.97	<.001	45.17 ± 3.29	<.001
		12	5– 6	90	55.47 ± 1.27	"	51.77 ± 1.66	"
Deer mice (W-M)	♂	10	16–18	0	86.91 ± 3.88		79.91 ± 3.43	
		10	"	65	69.96 ± 3.35	<.01	64.40 ± 2.04	<.01

* Mean ± stand. error of mean. † Time = Hr between inj. and autopsy.

P = Probability based on "Student"-Fisher t test of significance of differences between means. AH = Anterior hypothalamus. PH = Posterior hypothalamus.

Amines: None of the amines tested produced significant changes in HSD. These experiments employed, in rats: 1-epinephrine bitartrate (0.2 mg/kg, 18-20 hr; 2.5 mg/kg, 5-6 hr), 1-3, 4-dihydroxyphenylalanine (6-8 mg/kg, 4-6 hr), 5-hydroxytryptophan (18 mg/kg, 5-6 hr), tyramine monohydrochloride (40 mg/kg, 20 or 6-7 hr); in deer mice (SB-C): 1-epinephrine bitartrate (0.6 mg/kg, 18-20 or 6-7 hr, controls untouched).

Discussion. It appears unlikely that reduction of HSD following injection of PIH is due to increased levels of hypothalamic norepinephrine or serotonin, since our experiments with other monoamine oxidase inhibitors were negative even when precursors of these amines were administered. Nor can this effect of PIH be related readily to general central nervous system excitation since amphetamine did not produce decreased HSD and since in hamsters PIH produced severe agitation without significantly influencing HSD. Our surveys indicate that PIH reduces succinic dehydrogenase activity in some other areas of rat's brain as well. It should be emphasized that doses (mg/kg) of PIH which significantly decreased HSD in the rat are higher in our studies than the reported doses effective in monoamine oxidase inhibition and central nervous system stimulation in investigated mammalian species.

Amines that are known either to reduce "brain" succinic acid oxidation (O_2 uptake measured) *in vitro* (tyramine) or to stimulate posterior hypothalamic respiration and aerobic glycolysis *in vivo* (epinephrine) were not effective in modifying HSD *in vivo* in our experiments. This suggests that certain of the aerobic oxidative enzyme systems may be more sensitive than the succinic dehydrogenase system to the effects of these amines.

Summary. Succinic dehydrogenase activity of anterior and posterior hypothalamic areas (HSD) was measured by the reduction of a tetrazolium salt to its colorimetrically measurable formazan. The potent monoamine oxidase inhibitor, beta-phenylisopropylhydrazine, significantly reduced HSD activity in both anterior and posterior areas of adult rats and deer mice but not of hamsters. Other monoamine oxidase inhibitors, epinephrine and several other amines were without significant effects on HSD.

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