longed period. Animals prepared in this manner were able to tolerate for several weeks, without symptoms, a daily dose which would normally kill all rats in a few days. No gross or microscopic pathological changes were seen in the organs of the rats examined in this study.

1. Schrader, G., *The Development of New Insecticides,* Final Report, British Intell. Object. Sub-Comm., 1947, No. 714, 63, cited in Ripper, Greenslade and Hartley(2).

2. Ripper, W. E., Greenslade, R. M., and Hartley, G. S., *Bull. Entomological Res.,* 1950, v40, 481.

3. Heath, D. F., Lane, D. W. J., and Llewellyn, M., *J. Sci. of Food and Agriculture,* 1952, No. 2, 60.

4. DuBois, K. P., Doull, J., and Coon, J. M., *J. Pharm. and Exp. Therap.,* 1950, v99, 376.

5. Rider, J., Schulman, S., Richter, R. B., Moeller, H. C., and DuBois, K. P., *J.A.M.A.,* 1951, v145, 967.

6. Gregory, L., Futch, E., and Stone, C. T., *Am. J. Med.,* in press.

7. DuBois, K. P., and Mangun, G. H., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 137.

Received October 20, 1952. P.S.E.B.M., 1952, v81.

In vivo Inhibition of Liver and Brain Monoamine Oxidase by 1-Isonicotinyl-2-Isopropyl Hydrazine.* (19910)

E. A. ZELLER AND J. BARSKY.

From the Department of Biochemistry, Northwestern University Medical School, Chicago, Ill.

It has been shown that 1-isonicotinyl-2-isopropyl hydrazine (IIH) inhibits *in vitro* the monoamine oxidase of liver and brain of 6 different species(1,2). The inhibition is marked at concentrations as low as 0.2×10^{-5} molar. The question thus arose as to the possible occurrence of such a reaction *in vivo,* with the consequent production of symptoms as observed in the therapeutic application of IIH.

Studies on the cellular localization of monoamine oxidase reveal the enzyme activity to be largely present in the mitochondria(3-5). The addition *in vitro* of IIH to a mitochondrial suspension results in the disappearance of the monoamine oxidase activity. There is no restoration of activity by dialysis for 3 hours. It seemed then, that the administration of IIH to intact animals, followed by the isolation of mitochondria from tissues for the determination of monoamine oxidase activity would provide an indication as to whether the enzyme is inhibited *in vivo.*

Procedure. 1-Isonicotinyl-2-isopropyl hydrazine phosphate (IIH)† and isonicotinic acid hydrazide (INH)† dissolved in M/15 phosphate buffer pH 7.2 were injected subcutaneously into adult, male and female rats (Sprague-Dawley) and guinea pigs. Two hours later the animals were sacrificed by decapitation or by intravenous injection of air. The brain and liver were immediately removed, washed and chilled. Mitochondria were isolated by centrifugal fractionation of 1:10 tissue homogenates in 0.88 M sucrose solution according to the method of Hogeboom *et al.*(6). The characteristic morphology of the mitochondria was confirmed by phase microscopy. The determination of monoamine oxidase activity was carried out by the conventional manometric procedure for the estimation of oxygen uptake, and by microdiffusion analysis for the estimation of ammonia production. The incubation system contained, in a final volume of 2 ml, homogenate or washed mitochondria suspended in M/15 phosphate buffer pH 7.2 and 0.01 M tyramine hydrochloride (F. Hoffmann-La Roche); oxygen was employed in the gas phase. The enzyme suspensions per vessel were equivalent to 0.1 g fresh tissue for liver homogenate, 0.2 g original tissue for liver mitochondria and 0.33 g grey cortical tissue for brain mitochondria. The incubation was

* This work has been aided by a grant from the Multiple Sclerosis Foundation of Illinois.

† The compounds used were obtained from Hoffmann-La Roche, Inc., Nutley, N. J. IIH = Marsilid, INH = Rimifon, and from Bristol Laboratories, Syracuse, N. Y. (INH).

TABLE I. Effect of *In Vivo* Administration of Isonicotinic Acid Hydrazide (INH) and 1-Isonicotinyl-2-Isopropyl-Hydrazine (IIH) on Monoamine Oxidase of Rats.

Inhibitor, mg	Oxygen uptake, μmoles	Ammonia production, μmoles
	Liver homogenate	
0	90.5	119
IIH 10*	0	6
IIH 20	0	6
INH 10†	95.5	115
	Liver mitochondria	
0	34	40
IIH 10	1	1.5
IIH 20	3.3	2
INH 10	35.8	41
	Brain mitochondria	
0	12.9	14.4
IIH 10	4.8	4.8
IIH 20	1.5	1.5
INH 10	13.9	14.6

* 10 mg IIH = .036 millimole/rat = to .1 millimole/kg body wt.
† 10 mg INH = .073 millimole/rat = to .29 millimole/kg body wt.

TABLE II. *In Vitro* Effect of Hydrazides on Monoamine Oxidase of Guinea Pig Liver Mitochondria.

Inhibitor	Oxygen uptake, μmoles	Ammonia production, μmoles
0	46	53.5
.1 millimolar IIH	20.5	23
1 " INH	45.5	59.5

TABLE III. Effect of *In Vivo* Administration of 1-Isonicotinyl-2-Isopropyl-Hydrazine on Monoamine Oxidase of Guinea Pigs.

Inhibitor, mg	Oxygen uptake, μmoles	Ammonia production, μmoles
	Liver homogenate	
0	116	125
IIH 25*	7	4
	Liver mitochondria	
0	43.3	52
IIH 25	.5	1
	Brain mitochondria	
0	6.9	8.7
IIH 25	.9	1.2

* Equivalent to .18 millimole/kg body wt.

carried out at 38°C for 2 hours, after which 1 ml of the reaction mixture was transferred to Conway diffusion vessels. For final values, blank determinations were subtracted from those for the reaction mixture. The results are presented in the form of values per g fresh tissue for the 2-hour incubation period. Each set of values represents a mean of determinations on 2 animals.

Results. The administration of IIH leads to a nearly complete disappearance of monoamine oxidase activity of liver homogenate and mitochondria, and of brain mitochondria. INH, which even at 10^{-3} molar concentration *in vitro* has slight or no effect on liver(1) and brain monoamine oxidase(2) of the rat, also produces no apparent inhibition of the enzyme when administered *in vivo* (Table I).

Since no data on the *in vitro* effects of IIH and INH on guinea pig monoamine oxidase have been reported previously, typical values are presented in Table II.

The mitochondrial monoamine oxidase of guinea pig liver seems to be less sensitive toward IIH than that of the rat, which under identical conditions, is more completely inhibited. Nevertheless, after injection of IIH into guinea pigs, the enzyme activity again disappeared (Table III).

Discussion. Inasmuch as monoamine oxidase activity disappears from homogenates as well as from mitochondrial preparations. it is unlikely that the loss from mitochondria could be attributed to a change of the distribution of the enzyme among the different cell components, but points rather to an inactivation of the enzyme "in loco" by IIH. The observations indicate, therefore, that IIH not only enters the cell but also penetrates the mitochondria.

The elimination of monoamine oxidase activity *in vivo* should prove of value in the investigation of the physiological function of the enzyme, particularly with regard to its possible role in the degradation of epinephrine and nor-epinephrine. IIH may well become of the same importance for the study of the adrenergic system as has eserine in the analysis of the cholinergic system. Significantly, both types of brain cholinesterase are uninfluenced by the hydrazides(2). The above results may also offer an explanation for the sympathetic and general mental stimulation observed after administration of iso-

nicotinyl-hydrazides to patients and animals (7), in that the blocking of monoamine oxidase activity could conceivably lead to an accumulation of sympathomimetic amines with ensuing effects on the autonomic nervous system.

Summary. After the subcutaneous injection of 1-isonicotinyl-2-isopropyl hydrazine (IIH) into rats and guinea pigs, the monoamine oxidase activity of liver homogenates, liver mitochondria, and brain mitochondria is almost completely inhibited. The probable significance of such an inhibition is indicated.

1. Zeller, E. A., Barsky, J., Fouts, J. R., Kirchheimer, F. A., and Van Orden, L. S., *Experientia,* 1952, v8, 349.

2. Zeller, E. A., Barsky, J., Berman, E. R., and Fouts, J. R., *J. Pharm. Exp. Therap.,* 1952, in press.

3. Schweppe, J. S., Zeller, E. A., and Higgins, G. M., *Proc. Mayo Clinic,* 1951, v26, 371, footnote p. 374.

4. Cotzias, G., and Dole, V. P., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 157.

5. Hawkins, J., *Biochem. J.,* 1952, v50, 577.

6. Hogeboom, G. H., Schneider, W. C., and Pallade, G. E., *J. Biol. Chem.,* 1948, v172, 619.

7. Selikoff, I. J., Robitzek, E. H., and Ornstein, G. G., *Quart. Bull. of Sea View Hosp.,* 1952, vXIII, 17.

Received October 20, 1952. P.S.E.B.M., 1952, v81.

Dual Infection of Dogs with Distemper Virus and Virus of Infectious Canine Hepatitis.* (19911)

JAMES H. GILLESPIE, JAMES I. ROBINSON, AND JAMES A. BAKER.

From the Veterinary Virus Research Institute, New York State Veterinary College at Cornell University, Ithaca, N. Y.

Comparative studies(1) have shown that the illness produced by distemper virus resembles clinically the illness produced by the virus of infectious canine hepatitis. Both diseases were believed originally to be only one disease which was caused by the distemper virus, one of the earliest viruses isolated(2). Recent recognition of infectious hepatitis as a clinical entity(3) caused by a specific virus that produces characteristic intranuclear inclusion bodies, especially in hepatic cells, has now made possible the differentiation between this entity and that of distemper by histological studies. Shortly after the virus of infectious hepatitis was reported, mention was made of finding inclusion bodies of both distemper and infectious hepatitis in a dog that died from an illness attributed to distemper virus(4). Both distemper and infectious hepatitis have a high incidence rate(3,5). Following the report of inclusion bodies of both viruses in the same dog, further clarification seemed desirable. Studies were, therefore, undertaken in order to determine whether the two distinct viruses could infect dogs simultaneously, whether there would be discernible interference, and what the effects might be of such a concurrent infection. The results are reported herewith.

Experimental infection in dogs. Production. Puppies from 9 to 16 weeks of age were used and were obtained in litters of 3 or more. Prior to inoculation each litter was placed in an isolation unit and observed for at least a week for any sign of illness. During this period, temperatures and total leukocyte counts were taken daily. In instances where any puppy of a litter showed a temperature exceeding 39.5°C or a leukocyte count that deviated from the normal range, the entire litter was rejected. After the initial period of observation, puppies of each litter were divided into 3 groups and each group was placed in a separate isolation unit before inoculation. Each dog in 1 group was inoculated intravenously with 1 ml of a suspension in saline of spleen from a dog that had been given a strain of distemper virus originally obtained

* This investigation was supported in part by Swift and Co., and by a research grant from the National Institutes of Health, Public Health Service.