hold in the mucosa of the naso-pharynx. It is further reasoned that the M protein is then primarily responsible for the ultimate course of the infection as is the case with streptococcal cells introduced intraperitoneally. Facts tending to support this hypothesis are: a) The dissimilarity of aerosol infectivity and intraperitoneal virulence of a strain as shown by Coburn(5), b) failure of hyaluronidase treatment to appreciably modify virulence of intraperitoneally injected streptococci(4) and c) as shown by this study, the apparent non-infectivity by the aerosol route of streptococci producing hyaluronic acid but not detectable M protein, or of cells producing M protein but not detectable hyaluronic acid.

Recently Foley *et al.*(6) have reported that virulence of group A streptococci was related to resistance to surface phagocytosis and that this anti-phagocytic capacity was determined by the concomitant presence of large hyaluronic acid capsules and M protein production. Enzymatic destruction of either of these substances resulted in a marked and similar increase in phagocytosis of streptococcal cells by rat leukocytes. It was the opinion of these authors that capsular gel, as well as M protein, plays a significant role in streptococcal pathogenicity. The evidence obtained from our experiment supports their opinion.

Lastly, although there seems to be good evidence that production of HA is essential for aerosol infection, the cause for the high level of HA in broth supernatants of certain highly mouse infective strains has yet to be investigated. Experiments are in progress to determine whether formation of HA is inversely related to hyaluronidase produced.

Summary. Of the 16 group A streptococcal strains tested, only 9 exhibiting type-specificity and production of hyaluronic acid possessed capacity to induce aerosol infections in mice. Conversely, the remaining 7 strains which lacked either one or the other of these attributes failed to demonstrate infectivity by aerosol challenge. A statistically significant relationship between HA production and mouse infectivity was observed.

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Specific Liver and Brain Monoamine Oxidase Inhibition by Alkyl- and Arylalkylhydrazines.* (25660)

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Inhibition of the monoamine oxidase (MAO) enzyme of the brain has been demonstrated to result in an increase in brain levels of serotonin (5-hydroxytryptamine) and the catecholamines(1). This increase is believed to be the mechanism by which iproniazid (1 - isonicotinyl - 2 - isopropylhydrazine, Marsilid) exerts its psychic stimulant action. Since these earlier findings with iproniazid other hydrazine derivatives have been investi-

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gated for similar anti-MAO activity. Among those tested, the compound β-phenylisopropylhydrazine (PIH)† has been found to be an extremely potent inhibitor(2). In addition, Gogerty and Horita(3) demonstrated that PIH administered subcutaneously in graded doses to rats displayed greater brain than liver MAO inhibition, indicating a greater selectivity of the agent for brain tissue. In contrast iproniazid was found to exert an opposite pattern of selectivity; *i.e.*, liver MAO was more effectively blocked, and only by raising the dosage was the brain enzyme affected. These findings were of interest in view of recent reports concerning the possible hepatotoxic effects of prolonged therapy with iproniazid (4,5). The present paper is concerned with investigations to explain the difference between PIH and iproniazid in their relative anti-MAO actions of brain and liver *in vivo*. During these studies a number of related hydrazine derivatives have been employed, and evidence of a relationship between chemical structure and brain MAO inhibition has been found.

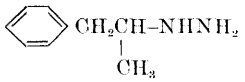
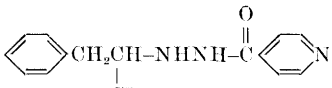
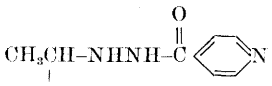
Methods and materials. Male Sprague-Dawley rats weighing 200-300 g were used as the experimental animals. All drugs were injected subcutaneously 2 hours prior to sacrifice of the animals. Livers and brains were rapidly removed, ground with Teflon homogenizers and made up to concentrations of 20% and 33% respectively. All procedures were carried out in an ice bath. One milliliter aliquots of the homogenates were incubated with 4 μmoles of serotonin creatinine sulfate and 0.5 M phosphate buffer at pH 7.0. Total volume of the incubation mixture was 3 ml. All incubations were carried out in a constant temperature shaker at 38°C for 30 minutes (liver) or 60 minutes (brain). Residual serotonin of these mixtures were then extracted and assayed colorimetrically using the nitrosonaphthol method described by Udenfriend *et al.*(6). Degree of MAO inhibition was calculated on ability of the drug to decrease rate of serotonin breakdown under conditions employed. The compounds investigated are shown in Tables I and II. Except for methylhydrazine (Eastman Chemicals) and iproniazid (Hoffmann-LaRoche, Inc.) the hydrazine derivatives were kindly supplied by Dr. J. H. Biel of Lakeside Laboratories, Inc.

Results. In the first series of experiments 4 compounds were studied. Their chemical structures are seen in Table I. In addition to PIH and iproniazid the compounds isopropylhydrazine and l-isonicotinyl-2-phenylisopropylhydrazine (isonicotinyl-PIH) were used. PIH showed marked brain MAO inhibition at a dose of 2.5×10^{-6} moles/kg while liver MAO activity was inhibited only to the extent of 25% (Table I). A similar trend was seen at both lower and higher dose levels. In the iproniazid treated rats a greater inhibitory effect of liver enzyme was seen at all dose levels used. Even at doses as high as 10^{-5} moles/kg when liver MAO was inhibited some 75%, brain MAO showed essentially no inhibition. When brains and livers from animals pretreated with isopropylhydrazine and isonicotinyl-PIH were assayed, both agents had produced a greater blockade of liver than of brain MAO. Isopropylhydrazine was found to be a highly potent inhibitor, showing potencies equal to that of PIH; however the pattern of brain and liver inhibition was reversed as compared to PIH, *e.g.*, whereas PIH exerted a 76% brain and 25% liver MAO inhibition at 2.5×10^{-6} moles/kg, isopropylhydrazine exerted a 25% brain and a 73% liver MAO inhibition at the same dosage.

These results indicated that the aromatic structure of PIH was important for selectivity for brain MAO *in vivo*. This led to the study of other alkyl and arylalkylhydrazine derivatives, methylhydrazine, benzylhydrazine, ethylhydrazine and β-phenylethylhydrazine. The chemical structures of these compounds are seen in Table II. The purpose of the second series of experiments was to demonstrate the influence of the benzene ring on brain and liver MAO inhibitory effects by the hydrazine compounds. The results are shown in Table II. Both benzyl- and β-phenylethylhydrazine exhibited much greater brain MAO inhibition as compared with the liver enzyme, while their alkyl congeners showed the opposite picture, supporting the results obtained with PIH and isopropylhydrazine. Although the brain-liver

† *Beta*-phenylisopropylhydrazine (PIH) is compound JB-516 (Catron), Lakeside Labs.

TABLE I. Liver and Brain Mao Inhibition *In Vivo* by Some Hydrazine Compounds.

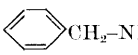
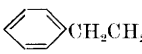
	Dose (moles/kg)	% inhibition Brain	Liver
 PIH	1×10^{-6} (8)	48 ± 9	10 ± 2
	2.5 × " (6)	76 ± 18	25 ± 6
	5 × " (4)	98 ± 3	84 ± 3
 Isonicotinyl-PIH	1×10^{-5} (6)	2 ± 3	79 ± 11
	5 × " (6)	49 ± 4	100 ± 0
$CH_3CH-NHNH_2$ | CH_3 *Isopropylhydrazine*	1×10^{-6} (2)	13 (4,22)	43 (29,57)
	2.5 × " (5)	25 ± 5	73 ± 15
	5 × " (2)	48 (64,33)	98 (96,100)
 Iproniazid	1×10^{-5} (5)	6 ± 6	74 ± 13
	5 × " (2)	58 (53,64)	100 (100,100)

Figures in parentheses under dose indicate No. of animals tested at each dose level. % inhibitions are mean values ± their stand. dev. Where n = 1 or 2, actual % inhibitions are placed in parentheses following mean values.

MAO inhibitory pattern of these compounds was related to presence of the benzene ring there was no such relationship in their potencies. Benzylhydrazine was much more effective than its alkyl congener methylhydrazine, while ethylhydrazine showed about a 10-fold greater potency than β-phenyl-ethylhydrazine. In contrast to either of the above pairs both β-phenylisopropylhydrazine (PIH) and isopropylhydrazine exhibited about the same degree of *in vivo* potencies.

Discussion. Gogerty and Horita(3) demonstrated that PIH exerted a more effective inhibition of brain than of liver MAO in the

TABLE II. Liver and Brain Mao Inhibition *In Vivo* by Some Hydrazine Compounds.

	Dose (moles/kg)	% inhibition Brain	Liver
 Benzylhydrazine	2.5×10^{-6} (5)	50 ± 8	21 ± 4
	5 × " (7)	76 ± 13	34 ± 8
	7.5 × " (6)	95 ± 7	71 ± 5
CH_3-NHNH_2 Methylhydrazine	1×10^{-4} (5)	33 ± 5	48 ± 3
	2.5 × " (2)	63 (66,59)	69 (74,64)
	5 × " (1)	72	93
 Phenylethylhydrazine	1×10^{-5} (7)	58 ± 16	14 ± 9
	2.5 × " (4)	96 ± 6	38 ± 9
$CH_3CH_2-NHNH_2$ Ethylhydrazine	2.5×10^{-6} (2)	12 (18,6)	25 (25,25)
	5 × " (5)	35 ± 11	58 ± 7
	1 × " (5)	64 ± 13	86 ± 3

Figures in parentheses under dose indicate No. of animals tested at each dose level. % inhibitions are mean values ± their stand. dev. Where n = 1 or 2, actual % inhibitions are placed in parentheses following mean values.

intact rat, while iproniazid showed an opposite pattern of inhibition. There are at least 2 possible mechanisms to explain this phenomenon of tissue or organ selectivity by these 2 drugs. The first is that the brain and liver MAO are different enzymes and that they differ in their affinities for drugs. This is, however, unlikely since there is no evidence that brain and liver MAO are different enzymes(7). The fact that in *in vitro* systems both brain and liver MAO are inhibited by iproniazid(8) or PIH(2) suggests that the enzymes are not markedly different. The second possibility is that the chemical structures of the drugs are sufficiently different to permit a different distribution to the various tissues. The brain is especially resistant to passage of certain types of agents because of its well-known but poorly-understood blood-brain barrier. This study supports the view that the chemical structures of the MAO inhibitors are important not only for potency but also for their abilities to exert more or less selective effects on brain MAO.

Upon finding the difference between iproniazid and PIH with respect to brain-liver MAO inhibition *in vivo*, we examined the isonicotinyl derivative of PIH (isonicotinyl-PIH) for its MAO effects. As reported earlier this compound was a much weaker agent than PIH but somewhat more potent than iproniazid(9). In the intact animal it also displayed a greater effect on liver than on brain MAO, thus resembling iproniazid. This finding suggested that the isonicotinyl grouping hindered passage of isonicotinyl-PIH or iproniazid into the brain. When the compound isopropylhydrazine was tested, however, it was found that it also showed a much greater effect on the liver MAO, and brain effects were quite small until much larger doses were used. From the experiments on these 4 compounds, the following has been concluded: (1) iproniazid is composed of 2 structural components which tended to prevent ready access to the brain cells, *i.e.*, the isonicotinyl and the isopropylhydrazine groups; (2) the isonicotinyl group not only hinders brain accessibility but also reduces potency of the compound; (3) the benzene ring of PIH is essential for the drug to exhibit brain selectivity.

The fact that the benzene ring is important for these compounds to exhibit brain selectivity is seen in the experiments with the alkyl and arylalkylhydrazines. In all instances aryl derivatives exerted greater brain than liver MAO inhibition, while alkyl compounds exerted more marked effect on the liver enzyme. It is possible that the aryl compounds, because of the presence of the benzene ring, are more lipoid soluble and therefore pass more readily across the blood-brain barrier. The benzene ring, however, does not alone influence ability of these compounds to exhibit brain selectivity. The presence of certain other groupings may prevent this effect, *e.g.*, with isonicotinyl-PIH, where the isonicotinyl moiety markedly decreased the preferential brain MAO inhibiting property of PIH. This is also true with substitutions of other chemical groupings on the ring, the side chain or the terminal hydrazine group (unpublished results). Of the numerous hydrazine compounds studied thus far it appears that the optimum structure for more selective inhibition of brain MAO *in vivo* consists of an unsubstituted benzene ring, a 2 carbon side chain and an unsubstituted terminal hydrazine group. An alpha methyl group does not affect the brain selectivity, but in fact increases potency of the MAO inhibiting property.

These findings may be of clinical significance since higher brain selectivity of the MAO inhibitors would be desirable for therapy of central nervous system disorders. Compounds such as iproniazid or isopropylhydrazine which act more effectively against liver MAO would thus be expected to exert greater peripheral effects, and for adequate central effects to be produced much higher doses would be required. It is possible that recent reports concerned with hepatotoxic actions of iproniazid during treatment of depression(4,5) may be the result of an excessive effect of this drug on the liver.

Summary. Ability of several hydrazine derivatives selectively to inhibit liver or brain monoamine oxidase (MAO) in the intact rat has been compared. Of those studied, the compounds possessing an unsubstituted arylalkylhydrazine structure, such as benzyl hydrazine, β-phenylethylhydrazine, and β-phenyl-

isopropylhydrazine showed greater brain than liver MAO inhibiting properties. Substitution on terminal hydrazine with an isonicotinyl group reversed the brain-liver MAO inhibiting relationship. Compounds lacking the benzene ring as in methyl-, ethyl-, and isopropylhydrazine also showed greater liver than brain MAO inhibiting effects. It is concluded that the benzene ring of the unsubstituted aryl-alkyl-hydrazines may aid in their passage into the brain.

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Influence of Local Hypothermia on Absorption from Isolated Intestinal Segments.* (25661)

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The transport of substances from the lumen of the small bowel to the blood stream under varying environmental conditions has always represented a fascinating physiologic problem. Some effects previously recorded are: interference with vascularity of an isolated loop of bowel slows absorption of strychnine(1); anoxemia increases absorption of water(2); drug-induced decrease of motility of the bowel impedes absorption of radiosodium and heavy water(4), vagotomy which reduces intestinal motility decreases glucose absorption from isolated jejunal loops, while sympathectomy has the opposite effect(5). The reduced intestinal motility(6) attending hypothermia suggested that the effect of hypothermia upon venous return and absorption from isolated canine intestinal loops should be assessed.

Methods and materials. The effect of hypothermia was determined on 1) venous outflow from closed jejunal and ileal loops, 2) strychnine absorption, and 3) removal of radioactive iodine (I^{131}) and radioactive iodinated serum albumin from isolated intestinal loops. Adult mongrel dogs weighing 6-10 kg, anesthetized with intravenous nembutal (30 mg/kg) were used in the study. Through a midline abdominal incision, a loop of ileum or jejunum was delivered through the wound. A 15 to 20 cm segment was isolated between 2 ties of umbilical tape. In the blood flow studies a Y cannula was placed in the mesenteric vein which drained the vascular arcades coming from the obstructed loop. The dogs were given heparin, 1.5 mg/kg. By placing clamps at appropriate sites, the flow could continue into the portal system without obstruction except when interrupted during timed periods (2 minutes) for measuring venous return. In the absorption studies, a polyethylene catheter was placed into the isolated segment through a small enterostomy opening for introduction of materials to be absorbed. When measurement of radioactivity in portal blood was needed, a catheter was threaded through the splenic vein into the portal vein, and 4 cc samples taken at appropriate intervals. In the normothermic (38.5°C) dogs, similar procedures were performed and the segment allowed to remain in

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