Cataractogenic and Lathyrogenic Effects of *N*-Phenyl-β-Hydrazinopropionitriles and Related Compounds in Rats[1] (34975)

WALDEMAR DASLER, HELEN L. S. WANG[2], THOMAS B. NORTON, AND F. DALE MCCOY[3]

Department of Biochemistry, The Chicago Medical School, Chicago, Illinois 60612

We have previously reported on the osteolathyrogenic properties of β-hydrazinopropionitrile (BHPN) and certain of its substitution products (1). In attempting to extend the study of this series of lathyrogens, we have succeeded in synthesizing and testing *N*-phenyl derivatives of BHPN containing (1) a phenyl radical on the terminal N atom of the hydrazino group; (2) a phenyl on the hydrazino N which is proximal to the carbon chain, and (3) the diphenyl derivative which contains a phenyl group on each of the hydrazino nitrogen atoms. These *N*-phenyl substitutions of BHPN as well as phenyl substitution on the amino nitrogen of β-aminopropionitrile (BAPN) caused marked reductions in lathyrogenicity of the parent compounds. Unexpectedly, one of these phenyl derivatives brought about lens opacities in the eyes of all rats consuming certain levels in their diets. A preliminary report of this finding has been made (2).

Materials. β-Aminopropionitrile (BAPN) fumarate was a gift from Abbott Laboratories, North Chicago, Illinois.

β-Hydrazinopropionitrile (BHPN), N′ methyl-BHPN and N^2-acetyl-BHPN were synthesized as previously described (1). The first two compounds were converted to their hydrochlorides.

N^1-Phenyl-β-hydrazinopropionitrile (N^1-Φ-BHPN), 3-(1-phenylhydrazino)-propionitrile, was prepared by reacting phenylhydrazine with acrylonitrile at room temperature in the presence of sodium methylate (3). The product was distilled *in vacuo* and then converted to the hydrochloride by adding alcoholic HCl to an ether solution: mp 133° (decomp). The neutralization equivalent and nitrogen analysis agreed with theoretical values, and the IR (infrared) spectrum was consistent with the above structure. The free base reacted with aldehydes and formed a hydrazone with *p*-nitrobenzaldehyde.

N^2-Phenyl-β-hydrazinopropionitrile (N^2-Φ-BHPN), 3-(2-phenylhydrazino)-propionitrile, was prepared by reacting phenylhydrazine with acrylonitrile on a steam bath in the presence of water made mildly acidic with acetic acid (3). The product was distilled *in vacuo* and converted to the hydrochloride in the same manner as the N^1-phenyl isomer. The hydrochloride did not melt, but decomposed very rapidly to form a new solid compound at 127°. The neutralization equivalent and nitrogen analysis agreed with theoretical values, and the IR spectrum was consistent with the above structure. The free base did not form a hydrazone with *p*-nitrobenzaldehyde.

N^1, N^2-Diphenyl-β-hydrazinopropionitrile (N^1, N^2-Φ_2-BHPN) was prepared by mixing hydrazobenzene with a slight excess of acrylonitrile in dioxane solution (3). The reaction was catalyzed by addition of a small amount of benzyltrimethylammonium methoxide in methanol. The product was crystallized by addition of water and purified by recrystallization from benzene-petroleum ether mixtures. The product melted at 119.5–123°. It did not form a hydrochloride. Its IR spectrum and results of nitrogen analyses were consistent with the expected structure.

N - Phenyl - β - aminopropionitrile (*N* - Φ - BAPN), β-anilinopropionitrile, was prepared by heating aniline and acrylonitrile in the presence of cupric acetate (4). The

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[2] Present address: Skin and Cancer Unit, New York University School of Medicine 10016.

[3] Present address: Hodag Chemical Corp., Skokie, Illinois 60076.

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hydrochloride was prepared by adding alcoholic HCl to an ether solution of the free base. Nitrogen analysis and neutralization equivalent agreed with theoretical values, and the melting points of both the free base and the hydrochloride agreed with values in the literature (4–7).

Methods. Different amounts of chemicals under test were added to a basal control diet which consisted of finely milled Rockland Rat Stock Diet. Tests were carried out with male Holtzman rats weighing 55–65 g. Rats were examined frequently for lens opacities as well as for skeletal deformities and other symptoms of lathyrism. Femurs and mandibles were removed and examined for gross osteolathyritic changes when animals died or were sacrificed. As a separate check on lathyritic connective tissue changes produced by test compounds, hydroxyproline levels were determined in saline-soluble and insoluble collagen fractions of sponge biopsies from adult rats which were fed these substances. These were compared to similar determinations on sponge biopsies from pair-fed normal controls by a device which we call the "lathyrogenic index" (LI) (see Table I). The LI is a measure of the "collagen-solubilizing effect" of the agent at the level fed, to the extent that its numerical value exceeds 1.0. Hydroxyproline was taken as a measure of collagen. The techniques for the collagen fractionations have been previously described (1).

In our earlier work involving collagen fractionation (8) we prefed the lathrogenic diet 1 day before sponges were implanted in order that they be implanted into a "lathyrogenic environment." Subsequent work has shown this to be unnecessary. In fact, we have found that lathyrogenic compounds give rise to increased saline-soluble collagen fractions

TABLE I. Lathyrogenic and Cataractogenic Effects of Feeding BHPN and Related Compounds.

	Adult rats			Weanling rats	
Chemical fed (%)	Age of sponge implant (days)[a]	Days on diet	LI[b]	Lathyrism	Cataracts
.20 BAPN · fumarate	0	14	2.3		
	0	21	3.7	+	—
	19	10	2.3		
.15 BHPN · HCl	0	14	2.3		
	0	21	2.8	+	—
	19	10	2.3		
.50 N^2-acetyl-BHPN	18	14	2.5		
	18	14	2.9	+	—
	44	17	2.2		
.25 N^2-ϕ-BHPN · HCl	18	14	1.3		
	44	13	1.3	(+)[c]	+
	24	10	1.4		
.15 N^2-ϕ-BHPN · HCl	18	11	1.8		
.20 N^1-ϕ-BHPN · HCl	0	15	1.1	—	—
.25 N^1-ϕ-BHPN · HCl	0	13	1.1		
.20 N^1,N^2-ϕ_2-BHPN	0	14	0.9	—	—
	0	14	1.1		
1.0 N-ϕ-BAPN · HCl	0	14	0.9	—	—
.50 N^1-Me-BHPN · HCl	19	10	2.2	+	—

[a] Pre-experimental.

[b] LI = lathyrogenic index = S_E/S_N where S_E = % of total sponge collagen which is saline-soluble (exptl.), and S_N = % saline-soluble collagen in sponges from pair-fed normals.

[c] (+) = very slightly lathyrogenic.

in sponges which have been implanted long before the lathyrogen-containing diet is fed. For many of our screening studies of potential lathyrogens, it has indeed been advantageous to use animals with preimplanted sponges because many of the chemicals were quite toxic and it was desirable to eliminate the added trauma of surgery from rats receiving such toxic diets. Tests with rats having preimplanted sponges are included in this study.

Results. Of the many compounds which we have tested and which are chemically related to, or are derivatives of, the nitrile-containing lathyrogens, only N^2-Φ-BHPN produced cataracts in rats. Dietary levels of 0.5% of the hydrochloride were lethal; the animals lost weight from the beginning of the feeding and died within 10 days. Levels of 0.05% of the hydrochloride did not produce cataracts of lathyrism even though the animals were fed the diet for 15 weeks. There was some depression of growth rate at this level (Fig. 1) and all animals exhibited splenomegaly at autopsy. At dietary levels of 0.10–0.25%, N^2-Φ-BHPN·HCl produced cataracts in all surviving rats in 11–17 days when fed continuously. Cataracts appeared in both eyes of an animal almost simultaneously, *i.e.*, never more than 1 or 2 days apart, and within a few days the entire lenses became completely opaque. Often a transitory pale or cloudy appearance of the eye occurred a few days before cataract development. In the few animals sacrificed at this stage, this transitory cloudiness appeared to be extralenticular.

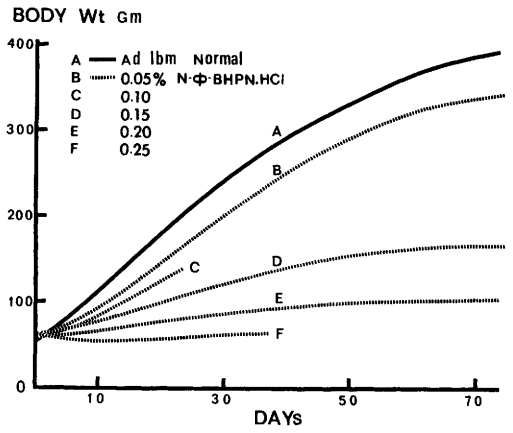

FIG. 1. Growth curves for rats receiving various levels of N^2-Φ-BHPN·HCl in their diets. All diets fed *ad libitum*.

Growth curves for weanling rats fed these diets are given in Fig. 1. All rats receiving N^2-Φ-BHPN·HCl suffered from splenomegaly. Some of the animals, especially those receiving dietary levels of 0.15–0.25%, had some of the symptoms commonly seen in rats receiving lathyrogenic diets, *viz.*, paraphymosis, incontinence, and slight spinal curvatures. The femur, when freshly removed usually had a soft excresence on the medial aspect at the insertion of the adductor longus muscle. This sometimes disappeared completely after air-drying, but often remained as a smaller ossified exostosis in the dried speciman. These evidences of a slight lathyrogenic action of this compound at 0.15–0.25% levels are confirmed by its moderate effect on raising saline-soluble collagen levels in sponge biopsies from adult rats (see Table I).

Adult rats (5–12 months of age) developed cataracts in 6–8 weeks when fed diets containing 0.15% N^2-Φ-BHPN·HCl.

None of the other structurally related compounds which were tested produced cataracts (Table I). Because phenylhydrazine is known to produce anemia and splenomegaly, it seemed possible that some of the effects of N^2-Φ-BHPN might be due to cleavage of the molecule with the formation of phenylhydrazine. However, phenylhydrazine hydrochloride did not produce cataracts when fed at various levels up to and including 0.2%, at which level most rats in the 50 to 70-g weight range lost weight and died within 2–3 weeks. Other aromatic hydrazines such as cyano- or nitrophenylhydrazine, or the hydrazinobenzoic or sulfonic acids, some of which are lathyrogenic (9), also had no cataractogenic activity.

Recovery from cataracts resulting from feeding 0.15% N^2-Φ-BHPN·HCl was extremely slow. When rats were transferred to normal Rockland diet immediately upon appearance of lens opacity, the lens first became completely opaque—usually within 24 hr. After some time, there then was a very

slow regression in the size of the opacity, but even after 15 months lenses still retained small opaque centers.

When young rats were transferred to a normal diet after consuming 0.15% N^2-Φ-BHPN·HCl for only a few days, changes apparently occurred during the initial feeding period which led to eventual cataract formation even though no more experimental diet was fed. This is shown in Table II. It is clear that individual susceptibility varied considerably. Two rats eventually developed cataracts in both eyes after only 3 days on the experimental diet, whereas one exceptionally resistant rat did not develop cataracts even when fed the experimental diet for 10 days (total period of observation 70 days). Most of these rats, like rats fed experimental diet continuously, developed cataracts in both eyes almost simultaneously. Striking exceptions were three rats in the group which received experimental diet for 6 days before being transferred to normal diet (Table II). These rats developed cataracts in one eye only (at 15, 16, and 32 days), while no opacity developed in the lens of the other eye over a 3-month period of observation.

TABLE II. Incidence of Cataract Formation in Rats Receiving Normal Diet After Short-Term Feedings at 0.15% N^2-φ-BHPN · HCl.

Exp. diet (days fed)	No. with cataracts/no. fed	Time of appearance of cataract (days)[a]
2	0/4	—
3	2/10	20–30 (24.8)
4	1/12	21 (21.0)
5	3/7	13–34 (21.0)
6	11.5/13[b]	12–32 (16.2)
7	10/12	12–26 (17.0)
8	10/10	14–20 (14.3)
10	9/10	10–20 (14.4)
12	4/4	14–15 (14.3)

[a] Earliest and latest days of cataract appearance after beginning experimental diet. Parentheses give mean values for all affected lenses in each group.

[b] All 13 rats in this group developed cataracts, but 3 rats developed cataracts in only one eye—these were counted as ½-rats. All other rats developed cataracts in both eyes or none at all (see text).

To our knowledge, there have been no previous reports of production of ocular lesions by lathyrogens or related compounds with the exception of those produced by the administration of β,β′-iminodipropionitrile (IDPN) (10–13). Depending somewhat on the species of experimental animal used, a variety of ocular abnormalities have been caused by IDPN. While clouding of the cornea was a common finding, in general these studies indicate no effect on crystalline lens. However, a more recent report by Paterson and Heath (14) stated that while majority of the lenses showed no morphological changes in rats, there was a faint opalescence near the anterior surface in a few cases. These results were obtained after a single subcutaneous injection with IDPN. On the other hand, others (15) make no mention of a cataractogenic action in rats after feeding 0.3% IDPN diets for up to 15 weeks.

Summary. Substitution of a phenyl group for a hydrogen on the amino nitrogen of β-aminopropionitrile (BAPN) eliminated the lathyrogenic activity of the parent compound. Phenyl substitution on either one of the hydrazino nitrogens of β-hydrazinopropionitrile (BHPN), or on both of these nitrogens, also largely destroyed the lathyrogenicity of BHPN. N^2-Φ-BHPN retained slight lathyrogenicity but produced cataracts in both eyes of all weanling rats in 11–17 days when fed as the hydrochloride at a level of 0.10–0.25% of the diet. Adult rats were also susceptible. When weanling rats were fed 0.15% N^2-Φ-BHPN·HCl for 3 or more days and were transferred to a normal diet before appearance of cataracts, many animals eventually developed cataracts, one as late as 29 days after removal of the cataractogenic diet. None of the other *N*-phenyl derivatives of BHPN or BAPN induced cataracts. N^1-methyl-BHPN and N^2-acetyl-BHPN also were not cataractogenic.

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1. Dasler, W., Norton, T. B., and McCoy, F. D., Proc. Soc. Exp. Biol. Med. 118, 719 (1965).

2. Wang, H. L. S., Dasler, W., and McCoy, F. D., Fed. Proc. 26, 800 (1967).

3. Pietra, S., Boll. Sci. Fac. Chim. Ind. Bologna **11**, 78, 83 (1953).

4. Heininger, S. A., J. Org. Chem. **22**, 1213 (1957).

5. Cookson, R. C., and Mann, F. G., J. Chem. Soc. **67**, (1949).

6. Butskus, P. F., J. Gen. Chem. **30**, 1816 (1960).

7. Bekhli, A. F., J. Gen. Chem. **21**, 86 (1951).

8. Dasler, W., Stoner, R. E., and Milliser, R. V., Metabolism **10**, 883 (1961).

9. Dasler, W., Norton, T. B., and McCoy, F. D., Sixth International Congress of Biochemistry, Abstracts **32**, 446 (1964).

10. Selye, H., Rev. Can. Biol. **16**, 1 (1957).

11. Selye, H., Amer. J. Ophthalmol. **44**, 763 (1967).

12. Forgacs, J., Arch. Ophthalmol. (Paris) **20**, 275 (1960).

13. Heath, H., and Rutter, A. C., Brit. J. Exp. Pathol. **47**, 116 (1966).

14. Paterson, R. A., and Heath, H., Exp. Eye Res. **6**, 233 (1967).

15. Hartmann, H. A., Lalich, J. J., and Akert, K., Neuropathol. Exp. Neurol. **17**, 298 (1958).

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