TECHNICAL PAPERS

Levo-Arterenol

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In 1910 Barger and Dale (*3*) speculated that the sympathetic effector substance might not be epinephrine but rather a catechol with a primary amine side chain. Efforts to demonstrate the existence of such a hormone and to identify it have continued uninterruptedly ever since. In recent years several investigators (*1, 2, 4, 5, 6, 8*) have conjectured that 3,4-dihydroxyphenylethanolamine (arterenol, norepinephrine, noradrenalin) might be the substance in question (sympathin E), although only indirect evidence has been adduced in support of this hypothesis. If the true hormone is arterenol, it might be supposed that it would be the *l*-isomer, rather than the *d*-isomer or the racemic mixture since *l*-isomers are generally more active.

Thus, the desirability of obtaining and studying *l*-arterenol has been appreciated for some time (*4, 5*). However, despite numerous efforts on the part of many investigators, arterenol has hitherto resisted resolution. Although Greer, *et al.* (*5*) indicated in 1938 that they were attempting the resolution, no report of success on their part has ever appeared. In our laboratory the problem has been under investigation for many years. Numerous trials at direct resolution by means of optically active acids failed, as did efforts at the resolution of regenerable derivatives of arterenol.

The purpose of this paper is to announce the successful preparation of *l*-arterenol by one of us (Tullar) and to present a brief description of its physical and physiological characteristics. Complete details will soon be published by us individually in appropriate journals.

The successful resolution of arterenol has now been accomplished by taking advantage of the fact that only the *l*-isomer forms a hydrated diastereoisomer with *d*-tartaric acid. From an aqueous solution of equimolar amounts of racemic arterenol and *d*-tartaric acid, the monohydrate of *l*-arterenol *d*-bitartrate crystallizes. The crude *d*-arterenol *d*-bitartrate from the mother liquor is purified by crystallization from 90% aqueous methanol. The optically active forms of arterenol are then readily obtained from these salts by treatment in aqueous solution with ammonium hydroxide.

Levo-arterenol hydrochloride is a white crystalline solid which is freely soluble in water. Solutions slowly oxidize under the influence of light and oxygen in a manner comparable to epinephrine hydrochloride. The melting points and optical rotations are given in Table 1.

As has been postulated from the activity of the *dl*-form (*7*), the *l*-isomer is more active in raising blood pressure than the *dl*- or *d*-form. In dogs under phenobarbital anesthesia this activity was found to be 164 ± 10% of that of *l*-epinephrine. This would give to *l*-epinephrine a dosage ratio of 0.61 for equal effect, a figure in excellent agreement with that of 1.2 for the racemic mixture, reported previously (*2, 7*). Dextro-arterenol has only 3–4% of the pressor activity of the levo, a ratio similar to that of the epinephrine isomers. The general

TABLE 1

	Physical Properties	
	M.p. (corrected) °C	$D^{25-9°}$
l-Arterenol	216.5–218	−37.3
d-Arterenol	215 –217	+37.4
l-Arterenol hydrochloride	146 –147	−39
d-Arterenol hydrochloride	146.8–147.4	+39

shape of the blood pressure curve of *l*-arterenol is like that of *l*-epinephrine, except that the secondary depressor phase is uncommon. The relative absence of stimulation of vasodilators is demonstrated also by the injection of Compound 933F (piperidinomethylbenzodioxan). At a time when *l*-epinephrine is reversed by this drug to a pure vasodilator action, *l*-arterenol still retains a small part of its pressor power and shows no reversal. In this respect, *d*-arterenol acts similarly to the *l*- in equivalent doses. Cocaine sensitizes the pressor response to *l*-arterenol to a considerably greater degree than to *l*-epinephrine.

TABLE 2

ACUTE TOXICITY OF INTRAVENOUSLY INJECTED ARTERENOL AND EPINEPHRINE FOR MICE AT THE END OF 24 HOURS

Compound (as base)	LD_{50} in group cages (mg/K)	LD_{50} in individual cages (mg/K)
l-Epinephrine	2.4 ± 0.2	2.5 ± 0.2
l-Arterenol	6.1 ± 2.0	20.5
d-Arterenol	70.7 ± 42.8	> 66

Excised strips of rabbit and guinea pig ileum are relaxed by *l*-arterenol to about the same degree as by *l*-epinephrine. However, on the nonpregnant rat uterus the *l*-arterenol is a relatively weak inhibitor, since it takes 30 times the concentration to reproduce the relaxation of the stimulated organ produced by *l*-epinephrine. These results indicate that *l*-arterenol can produce inhibitory effects in at least some organs, but that its spectrum of action differs from that of epinephrine.

The acute intravenous toxicity for rapid injection in mice is summarized in Table 2.[1] It was found that the

[1] The authors are indebted to J. O. Hoppe for the toxicity determinations.

toxicity of the *l*-arterenol is reduced when the animals are kept in individual cages, as shown in the last column of the table.

The arterenol toxicity curves are quite flat, as is indicated by the relatively large standard errors. These figures indicate that for equivalent pressor doses *l*-arterenol has a safety ratio (toxicity to pressor activity) which is approximately 4 times that of *l*-epinephrine. This is in agreement with the earlier results (*7*) on the racemic mixture.

Now that *l*-arterenol has been obtained and can be made available for physiological experimentation, it is expected that complete elucidation of its role in the mediation of sympathetic functions and application to therapeutics will follow promptly.

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Nitrogen Mustards in Fowl Leucosis[1]

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The recent reviews by Bodansky (*1*) and by Gilman and Philips (*3*) indicate that nitrogen and sulfur mustards elicit a variety of systemic pharmacological actions, chief of which is their ability, in some unexplained manner, to produce death of cells. The generalization is made that cellular susceptibility is related to proliferative activity. The action of the mustards on the blood-forming organs as reflected in the peripheral blood of both experimental animals and man results in a lymphocytopenia, granulocytopenia, thrombocytopenia, and moderate anemia. Because of the marked effects of the mustards on lymphoid tissue, coupled with the finding that actively proliferating cells are selectively vulnerable to the cytotoxic action of the mustards, therapeutic use of these compounds in the treatment of neoplasms of lymphoid tissue in fowls was undertaken. The use of nitrogen mustards in such diseases as Hodgkin's disease, lymphosarcoma, and leucemia in man was sufficiently encouraging to warrant studies with similar diseases of animals (*4, 6*).

Leucosis of fowls is a disease somewhat similar to leucemia of mammals. In fowls the disease is transmissible, and the causative agent is recognized as a filtrable virus. Several forms of the disease occur, depending upon the type of proliferating cells in predominance and upon the tissues involved. The various forms have been designated as lymphomatosis, which may be neural, visceral, ocular, or osteopetrotic and may be leucemic, subleucemic, or aleucemic; and leucosis, which may be erythroblastic or granuloblastic (*9*).

The birds used in these experiments were purchased as day-old chicks from a nearby hatchery and were infected artificially when they were from 1 to 2 weeks of age.

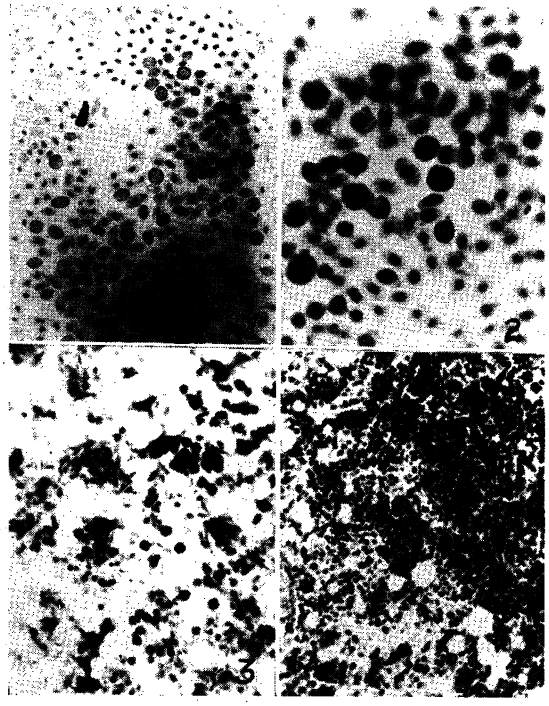

FIG. 1. (1) Photomicrograph of blood smear from chicken with hemocytoblastic leucosis, stained with Wright's stain (×368). (2) Same as (1) at higher magnification (×750). A marked reduction in numbers of these large immature cells was noticeable as early as 24 hrs following treatment with the nitrogen mustards. Note one cell in mitosis. (3) Photomicrograph of femoral marrow from a chicken 4 days after receiving a toxic dose of mustard (×368). Note depletion of lymphoid cells. (4) Photomicrograph of femoral marrow from a normal chicken of the same group and of the same age. Note hyperplasia in contrast to (3).

About equal numbers of the Barred Plymouth Rock and New Hampshire Red breeds were used. The Beltsville strain "A" leucosis virus was used throughout (*5*). When week-old chicks were injected intravenously or intraperitoneally with blood containing this strain of virus, they usually developed an acute form of erythrogranuloblastic leucosis in from 4 to 6 weeks. The injected birds that did not develop an acute form usually later developed either a chronic visceral form characterized by great enlargement of the liver, spleen, and other visceral organs or the nerve form characterized by cellular infiltration of nerves, and paralysis (*10*). The

[1] The nitrogen mustards were obtained through the courtesy of the Medical Division of the Army Chemical Center.