will depend upon the ability to bring adequate concentrations of the drug into contact with the tumor. As was the case with other drugs studied in human tumors,[5] individual tumor sensitivity to tubercidin varied greatly irrespective of tissue of origin or of histologic type.

Acknowledgment—The technical assistance of Miss Phyllis Farnsworth is greatly appreciated.

Division of Clinical Oncology and Department of Surgery, WILLIAM H. WOLBERG
Medical School, University of Wisconsin,
Madison, Wis., U.S.A.

REFERENCES

1. S. SUZUKI and S. MARUMO, *J. Antibiot. (Tokyo.)* **13A**, 360 (1960).
2. S. P. OWEN and C. G. SMITH, *Cancer Chemother. Rep.* **36**, 19 (1964).
3. C. G. SMITH, W. L. LUMMIS and J. W. GRADY, *Cancer Res.* **19**, 847 (1959).
4. G. ACS, E. REICH and M. MORI, *Proc. nat. Acad. Sci., (Wash.)* **52**, 493 (1964).
5. W. H. WOLBERG, *Cancer Res.* **24**, 1437 (1964).

Biochemical Pharmacology, 1965, Vol. 14, pp. 1925–1927. Pergamon Press Ltd., Printed in Great Britain.

The chick embryo as a biologic indicator: Structure–function relationship of sympathomimetic agents*

(*Received* 15 *March* 1965; *accepted* 16 *June* 1965)

MANY different compounds have been introduced as vasopressor agents over the years. This group includes both aromatic and aliphatic compounds of saturated and unsaturated linkages. A substituted amine group in the terminal position of the aliphatic chain is, however, a feature common to all agents in this group.

Epinephrine, norepinephrine, and phenylephrine have been previously shown to produce distinctive lesions in the chick embryo.[1] Application of 50 μg of either of the agents to the chorio-allantoic membrane induced cephalic hematoma and/or skin and extremity hemorrhage. The cephalic hematoma occurred within the subcutaneous mesenchyme of the head, cephalad to the optic lobes in the area of the epiphysis. Grossly, the lesion was elevated, blue-black, and occupied the greater portion of the dorsum of the head. Microscopically, the area of hemorrhage extended beneath the developing membranous bone of the skull and into the subdural space. Extremity hemorrhage in its most advanced stage resembled hemorrhagic infarction; its mechanism differed from that of the cephalic lesion.[1] Pretreatment of the embryos with cortisone resulted in complete inhibition of the cephalic hematoma. Cortisone did not inhibit skin and extremity hemorrhage induced by norepinephrine or phenylephrine.[1] The lesions were distinctive, reproducible, and evolved rapidly. This model has been suggested as a measure of the pharmacologic activity of the sympathomimetic agents on blood vessels.[1]

The purpose of this paper is to report an evaluation of additional vasopressor agents by means of this biologic system.

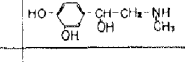
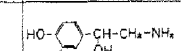
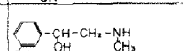
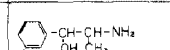
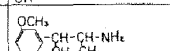
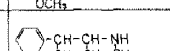
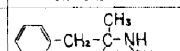
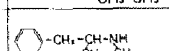
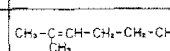
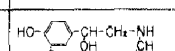
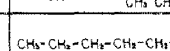
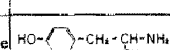
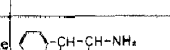
METHOD

The drugs were dropped directly upon the chorio-allantoic membrane of the 10-, 11- or 12-day chick embryos as previously described.[1]

RESULTS AND DISCUSSION

Table 1 lists thirteen adrenergic drugs and indicates the response of the chick embryo to them both before and after pretreatment with cortisone. Four of these drugs have been included in a previous report.[1] By observing the structural formulas it can be noted that those compounds with β-hydroxyl substitution of the side chain, a relatively free amine group on the α-carbon of the side chain, and one or two hydroxyl or methoxy groups attached to the aromatic ring produced lesions. Aliphatic compounds were inactive.

TABLE I

Drug Official Name (Trade Name)	Formula	Amount micro-grams	Effect on the Chick Embryo		Effect Following Pre-treatment with Cortisone		
			Cephalic hematoma	Skin & extremity hemorrhage	Prevention of all lesions	Prevention of cephalic hematoma only	Skin blanching
Epinephrine (Adrenalin)		50	present	present	yes	no	yes
Levarterenol (Norepinephrine) (Levophed)		50	present	present	no	yes	no
Phenylephrine (Neo-Synephrine)		125	present	present	no	yes	no
Metaraminol (Aramine)		50	present	present	no	yes	no
Methoxamine (Vasoxyl)		1000	present	present	no	yes	no
Ephedrine		5000*	no	no	-	-	-
Mephentermine (Wyamine)		750	no	no	-	-	-
Methamphetamine (Methedrine)		1000	no	no	-	-	-
Isometheptene (Octin)		5000	no	no	-	-	-
Isoproterenol (Isuprel)		50	no	no	-	-	-
Methylaminoheptane (Oenethyl)		2000	no	no	-	-	-
Hydroxyamphetamine (Paredrine)		500	no	no	-	-	-
Phenylpropanolamine (Propadrine)		3000	no	no	-	-	-

*Where large doses are indicated, these represent maximum amounts used after smaller doses were ineffective.

These experiments indicate that a particular molecular configuration was required to induce the morphologic lesions described, and that the presence of and extent of the lesions were reliable evaluations of the vascular response expected from these agents. The indicator lesions were: (1) cephalic hematoma and (2) extremity hemorrhage.

Several pertinent structure–function generalizations can be made. By comparing the formulas of metaraminol and phenylpropanolamine (Table 1) the importance of the hydroxyl group at the *meta* position of the aromatic ring is obvious and was the determining factor in rendering metaraminol active while phenylpropanolamine was inactive. Similar effects of metaraminol and phenylephrine indicate that monomethyl substitution of the terminal amine group is without significance in compounds with monohydroxyl substitution of the aromatic ring. The effect of these compounds was similar to norepinephrine, which possesses no methyl group on the side chain but has an additional hydroxyl group attached to the aromatic ring. On the other hand, epinephrine differs from phenylephrine structurally only by the presence of an additional hydroxyl group on the aromatic ring; yet the action of epinephrine differed from phenylephrine after pretreatment with cortisone.

Hydroxyamphetamine is characterized by monohydroxyl substitution at the *para* position of the aromatic ring but differs from metaraminol in that there is no β-hydroxyl substitution of the side chain; it had no demonstrable effect on the chick embryo and showed the importance of the β-hydroxyl substitution.

The only remaining drug of those tested that produced lesions in the chick embryo was methoxamine; this drug had a norepinephrine-like effect but differed from it structurally by 2,5-dimethoxy substitution of the ring instead of 3,4-dihydroxy substitution, indicating that other ring-activating groups might be substituted for the hydroxyl groups.

Isoproterenol differs from norepinephrine only by isopropyl substitution at the amine group, but it was an ineffective agent. This substance illustrated that isopropyl substitution on the amine group interfered with drug action.

In order for a drug to stimulate this toxic response of the vessels of the chick embryo it must have the basic structure of ethylamino benzene, β-hydroxyl substitution of the side chain, a degree of physical freedom of the amine group, and hydroxyl or methoxy ring substitution. The position of the latter groups did not seem to be important. If one observes the list of adrenergic drugs included in this report, those that have proved to be most useful clinically as cardiovascular stimulants are the ones that have met the molecular requirements listed above.

Since no proof is available that 10-, 11- or 12-day chick embryos possess stored catecholamines, this work probably does not indicate action of adrenergic drugs that function indirectly through release of such stores.

Astle and Shelton[2] state that with the exception of the halogens, the groups which direct the second substituent to the *ortho* and *para* positions activate the aromatic ring and that substitution occurs more readily than with benzene. Some of the common *ortho-para* orienting groups are —OH, —NH_2, —CH_3, —OCH_3, —NHR, and —NR_2; of these groups, the hydroxyl, methoxy, and amine groups are said to be most active. The halogens deactivate the ring but still direct *ortho* and *para* substitution. If the —OH or —OCH_3 substituted groups on the benzene ring of the "active" adrenergic drugs were replaced by halogens, the role of ring activity might be evaluated.

This model is suggested as a means of screening hypothetical sympathomimetic agents. If such a drug induces cephalic hematoma and hemorrhage in the extremities of 10-, 11-, or 12-day old chick embryo, a sympathomimetic effect may be indicated. Prevention of only cephalic hematoma by cortisone may indicate norepinephrine-like effect, while prevention of all lesions by cortisone may indicate epinephrine-like effect.

Departments of Pathology, ROBERT R. GATLING
St. Dominic-Jackson Memorial Hospital,
and University of Mississippi School of Medicine,
Jackson, Miss., U.S.A.

REFERENCES

1. R. R. GATLING, *Am. J. Path.* **40**, 113 (1962).
2. M. J. ASTLE and J. R. SHELTON, *Organic Chemistry*, 2nd ed., p. 431. Harper, New York (1959).

* Supported by United States Public Health Service Grant AM 04510-03.