larly with Normal Duck Serum (NDS) failed to develop arthritis. 2) Rabbits with a remote history of immunization against duck serum developed an accelerated arthritis within *one week* following i.a. injections of either DARKS or NDS. 3) Nonimmunized animals preconditioned with isotonic NaCl or KCl given instead of drinking water, developed accelerated arthritis within 2 to 4 weeks following i.a. injections not only of DARKS but of normal duck, calf or horse serum as well. 4) Circulating anti-duck serum precipitins always preceded development of arthritis induced by i.a. injections of duck serum. 5) The arthritic changes consisted of villous hypertrophy and infiltration of the synovium with lymphocytes and plasma cells. This was followed by fibrinoid necrosis of the synovium and articular cartilage and by formation in the joint cavity of an exudate of proteinaceous material, round cells and polymorphonuclear leukocytes.

The authors wish to acknowledge the assistance of Dr. Paul Brancato. They are grateful to Mr. Herbert A. Fischler for preparing the photographs and to Mrs. Renee Nakrinsky for typing the manuscript.

1. Teodoru, C. V., Volk, B. W., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 274.

2. Teodoru, C. V., *Fed. Proc.*, 1961, v20, part I.

3. Brancato, P., Teodoru, C. V., Volk, B. W., *ibid.*, 1961, v20, part I.

4. Teodoru, C. V., Saifer, A., Frankel, H., *Am. J. Physiol.*, 1959, v196, 457.

5. Mellors, R. C., *Analytical Cytology. Methods for Studying Cellular Form and Function.* 2nd Edition. McGraw-Hill Book Co., Inc., New York, 1959.

Received March 12, 1962. P.S.E.B.M., 1962, v110.

Effect of Phenylephrine on Responses of the Rabbit Aortic Strip to Isoproterenol.* (27552)

GERTRUDE D. MAENGWYN-DAVIES
(With the technical assistance of Barbara Stuelcken)
Department of Pharmacology, Georgetown University, Medical Center, Washington, D.C.

In the atropinized dog and cat the vasodepressor action of isoproterenol could be converted to a vasopressor effect by infusion of large amounts of sympathomimetic amines (1). Phenylephrine, one of the most potent amines studied, induced vasopressor responses as well as an increase of peripheral resistance and contractions of the nictitating membrane to isoproterenol. The responses to epinephrine remained essentially unchanged(2). *Pretreatment* with large doses of dichloroisoproterenol (DCI) did not alter isoproterenol vasomotor reversal by phenylephrine. Therefore, this phenomenon was attributed in part to a "sensitization" of contractile (alpha, 3) receptors.

Levy and Ahlquist(4) found that *pretreatment* by the alpha receptor blocking agent, dibenzchlorethylamine, inhibited isoproterenol vasomotor reversal and that DCI, administered after isoproterenol vasomotor reversal had been established, markedly reduced the pressor responses. This latter observation was further corroborated and extended in our laboratory. The original height of the pressor responses to isoproterenol could be reestablished by small additional doses of phenylephrine, administered during the duration of the DCI-blockade(5).

Isoproterenol vasomotor reversal by vasopressin was investigated by Nash, Boyajy and Manley(6). Following vasopressin, the increase in femoral arterial blood flow produced by isoproterenol was significantly reduced but not reversed. Blood pressure responses to epinephrine were significantly augmented and the effect of epinephrine on femoral arterial blood flow was reversed. These authors, therefore, concluded that "the reversal of isoproterenol and the potentiation of epinephrine seen in these studies can be explained on the basis of selective blockade of peripheral vaso-

*Supported by grant from Nat. Inst. of Neurol. Dis. and Blindness, U.S.P.H.S.

dilating receptors without a concomitant blocking of cardiac stimulation."

In view of these contradictory findings and interpretations of phenomena observed in the whole animal, it seemed of interest to determine whether or not phenylephrine could influence the responses to isoproterenol in the rabbit aortic strip which is known to contain both relaxing and contractile receptors.

Methods and materials. Strips of the aortae of 2.5 to 3.0 kg male rabbits were set up as described by Furchgott and Bhadrakom (7) and used according to the modification introduced in our laboratory(8). At the beginning of each experiment a graded dose-response curve to l-phenylephrine hydrochloride (PE; Winthrop Laboratories, Inc.) and l-isoproterenol bitartrate dihydrate (ISU; Sterling-Winthrop Research Inst.) was obtained by applying, once every 7 minutes, single doses of drug, each twice the amount of the previous one, permitting the tissue to contract for 3 minutes, as described previously(8,9). This procedure established the sensitivity of each individual strip and allowed the choice of a dose of PE contracting the strip to well below 50% of its maximum contraction height, as well as of graded doses of ISU from subcontractile concentrations to amounts which contracted the strip to about 25% of its maximum.

Two control doses of each drug given separately, were randomly alternated, followed by a single test dose of the identical concentrations of each drug given simultaneously. The contraction time was increased arbitrarily to 6 minutes and doses spaced 10 minutes apart, since the responses of the strip to the 2 compounds given simultaneously, in contrast to the immediate onset of contractions when each drug was given separately, showed a lag time of about one minute. Each test was repeated twice, or more often, in each strip and verified in at least 4 different strips. Relaxation or contraction of the strips is reported in mm (magnification 1:20). PE and ISU were freshly dissolved daily in glass distilled water and kept in an ice bath during use. Doses are expressed as μg of base per 30-ml bath. In some experiments, in order to mimic conditions in the whole animals which were pre-

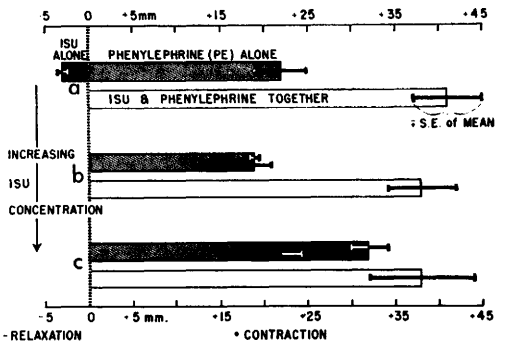

FIG. 1. Two separate bars for means of control contractions in mm to isoproterenol (black) and to phenylephrine (striped). Means of test responses to their mixture at identical doses in mm in a third bar (clear); ± stand. error of mean indicated by thin black lines. Bath fluid: Krebs-bicarbonate Ringer solution *without* atropine. (a) Dose of ISU, 13.34 μg; of PE, 1.119 ± 0.173 μg; No. of strips, 7. (b) Dose of ISU, 26.68 μg; of PE, 1.23 ± 0.148 μg; No. of strips, 6. (c) Dose of ISU, 53.36 μg; of PE, 1.168 ± 0.123 μg; No. of strips, 6.

treated with atropine(1,2), atropine sulfate (Merck and Co., Inc., 1.86 mg/liter) was incorporated into the bath fluid. The averages in each individual strip of the repeated control and test responses at each dose level were statistically evaluated by the t-test for paired experiments(10).

Results. 1. *Bath fluid: Krebs-bicarbonate Ringer solution without atropine.* A dose of 1.172 ± 0.145 μg of PE produced 34 ± 4% of the maximum contraction heights of all strips. Three doses of ISU, each double the amount of the previous one, were chosen, starting with a subcontractile dose. The highest dose of ISU caused a contraction (Fig. 1,c). The contractions generated by simultaneous addition of PE and ISU, when the latter was applied in either a subcontractile (Fig. 1,a; $p<0.001$) or in a borderline contractile dose (Fig. 1,b; $p<0.01$) were augmented significantly above a mere additive effect of the two drugs. When the contractile dose of ISU was applied together with PE, a mere additive effect of the 2 drugs was seen (Fig. 1,c; $p<0.3$) although the contraction height (65 ± 10% of maximum) remained well below the full contractility of each strip.

2. *Bath fluid: Krebs-bicarbonate Ringer solution with atropine.* Although the presence of atropine diminished the sensitivity of

TABLE I. Effect of l-Phenylephrine (PE) on 6-Minute Aortic Strip Responses to l-Isoproterenol (ISU) in Presence of Atropine Sulfate (18.6 mg/l of Bath Solution).

ISU dose, μg	Responses to ISU, mm	PE dose, μg	Responses to PE, mm	Responses to both drugs, mm	Difference in response	p
	Mean ± S.E.					
26.68 (5)*	− 3 ± .4	4.77 ± .55	+19 ± 2.5	+29 ± 4.9	13.1 ± 2.98	<.02
53.36 (11)	0	5.08 ± .92	+19 ± 2.3	+26 ± 3.0	7.7 ± 1.85	<.01
106.72 (12)	+ 5 ± 1.4	4.73 ± .93	+18 ± 1.8	+34 ± 3.1	8.33± 1.94	<.01
213.44 (6)	+12 ± 5.4	5.11 ± .9	+16 ± 3.2	+39 ± 5.9	11.2 ± 2.78	.01

* Figures in parentheses refer to No. of strips.

− denotes relaxation
\+ " contraction

the rabbit aortic strip about 4-fold, maximum contraction height was not depressed. Essentially the same observations were made in the presence of atropine as in its absence, except that the augmentation of responses to the drug mixture remained statistically significant even with low contractile doses of ISU (Table I). With either lower or higher doses of ISU than shown in Table I, an augmentation of responses did not occur.

Discussion. High concentrations of ISU contract the aortic strip to its maximum contraction height(9). Subcontractile doses of ISU inhibit contractions of the aortic strip produced by norepinephrine, epinephrine, histamine, serotonin or KCl(11). In contrast, subcontractile doses of ISU given simultaneously with contractile doses of PE produce a significantly higher contraction of the rabbit aortic strip than the mere additive response to each drug given by itself. In the whole animal norepinephrine did not reverse ISU; in fact, it inhibited ISU reversal by PE(12). The difference of ISU effects, depending on which contractile agent is being studied *in vitro*, and the parallelism of the observations in the aortic strip and in the intact animal indicate that PE alters the responses to ISU. As a working hypothesis it may be suggested that tissue permeability may be increased by PE and as a consequence, the effective ISU concentration reaching the contractile receptors may be augmented. Acetylcholine is thought to act as a contractile agent by altering cellular ion permeability(13,14,15) and atropine is a well established anticholinergic drug, which may show this pharmacological property because it decreases membrane permeability. In the aortic strip, in the presence of atropine, about a 4-fold increase in the doses of either ISU or PE is required to obtain the usual dose-response curve seen in non-atropinized preparations. Atropine enlarges the range of ISU concentrations to which responses are significantly augmented by PE. This further suggests that PE might influence membrane permeability.

In the aortic strip subcontractile doses of ISU could be converted into contractile doses by simultaneous application of PE. This phenomenon simulates isoproterenol vasomotor reversal in the whole animal and corroborates our previous conclusion, that an alteration of the responses of the peripheral vascular bed has a role in isoproterenol vasomotor reversal by PE. This statement should not be interpreted to mean an exclusion of the significant contribution of the heart.

In the intact animal responses to epinephrine are potentiated by doses of vasopressin which produce isoproterenol vasomotor reversal, whereas they remain essentially unchanged by doses of PE which elicit reversal of ISU. Thus an entirely different mechanism may be responsible for the vasomotor reversal of ISU by non-specific vasoconstrictor agents than by sympathomimetic amines.

Summary. 1. Simultaneous addition of l-phenylephrine (PE) and l-isoproterenol (ISU) to the rabbit aortic strip significantly augmented contractions above the mere additive responses produced by each drug alone. 2. Addition of atropine to the bath fluid permitted an increase of ISU dose range which, together with PE, produced such augmented contractions. 3. The findings are correlated with ISU vasomotor reversal in the whole animal.

I am indebted to Dr. Theodore Koppanyi for his interest and encouragement.

1. Walz, D. T., Koppanyi, T., Maengwyn-Davies, G. D., *J. Pharmacol. Exp. Therap.*, 1960, v129, 200.

2. Walz, D. T., Maengwyn-Davies, G. D., *ibid.*, 1960, v129, 208.

3. Ahlquist, R. P., *Am. J. Physiol.*, 1948, v153, 586.

4. Levy, B., Ahlquist, R. P., *J. Pharmacol. Exp. Therap*,. 1960, v130, 334.

5. Cowan, F. F., Koppanyi, T., Maengwyn-Davies, G. D., *Georgetown Med. Bull.*, 1961, v15, 65.

6. Nash, C. B., Boyajy, L. D., Manley, E. S., *Arch. Int. Pharmacodyn.*, 1961, v133, 433.

7. Furchgott, R. F., Bhadrakom, S., *J. Pharmacol. Exp. Therap.*, 1953, v108, 129.

8. Wurzel, M., Pruss, T. P., Weiss, W., Maengwyn-Davies, G. D., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 659.

9. Wurzel, M., Koppanyi, T., Pruss, T. P., Maengwyn-Davies, G. D., *Arch. Int. Pharmacodyn.*, in press.

10. Batson, H. C., *An Introduction to Statistics in the Medical Sciences*, Burgess Publishing Co., Minneapolis, Minn., 1956.

11. Pruss, T. P., Koppanyi, T., Maengwyn-Davies, G. D., *Arch. Int. Pharmacodyn.*, in press.

12. Maengwyn-Davies, G. D., Walz, D. T., Koppanyi, T., *ibid.*, 1961, v134, 61.

13. Thesleff, S., in *Structure and Function of Muscle*, Ed. Bourne, G. H., Academic Press, New York, 1960, v3, Chapt. I.

14. Born, G. V. R., Bülbring, E., *J. Physiol.*, 1956, v131, 690.

15. Burnstock, G., *ibid.*, 1958, v143, 165.

Received April 6, 1962. P.S.E.B.M., 1962, v110.

Preparation of Measles Antiserum in Horses. (27553)

E. B. BUYNAK, M. R. HILLEMAN, AND A. F. WOODHOUR
Division of Virus and Tissue Culture Research, Merck Institute for Therapeutic Research, West Point, Pa.

The need arose in our laboratory to prepare a large volume of measles immune serum for research purpose. This was accomplished simply and effectively by immunizing horses with measles virus emulsified in Freund's incomplete Arlacel A-mineral oil adjuvant. The present report summarizes the technics used and the findings.

Materials and methods. Virus. The Edmonston(1) strain of measles virus was obtained from Dr. John F. Enders and was received in the 29th passage in primary cell cultures of human amnionic membrane. The virus was carried in our laboratories in the Fogh and Lund(2) and in the Wistar Institute WISH(3) line cell cultures of human amnion. Virus which had been serially passed 10 times in our laboratory was used for immunization purpose. The infectivity titer of the virus used was $10^{-4.5}$ per 0.1 ml and the complement-fixing (CF) titer was 1:16. *Emulsification.* Equal volumes of the live measles virus and of the Arlacel-mineral oil mixture* were emulsified by ordinary force-mixing procedures. The Arlacel and mineral oil components were tested for toxicity by usual methods† prior to use. The emulsified vaccine was free of bacterial and mold contamination and was stored at 4°C in the refrigerator until used up to 4 weeks later. *Immunization.* The horses were ordinary draft animals of 11 to 17 years age and weighed 1000 to 1200 lb. Pre- and post-vaccination bleedings were in 4 liter amounts and were drawn into evacuated flasks *via* a trocar inserted into the jugular vein. Injections of aqueous vaccine were of 10-ml volume and were given into the lateral cervical muscles of the neck. The first dose of adjuvant vaccine given into the neck muscles caused stiffness and, thereafter, injections were made into the gluteal muscles. The dose of adjuvant vaccine consisted of two 10-ml injections given

* 10% Arlacel A, Atlas Powder Co., Wilmington and 90% Drakeol 6 VR, Pennsylvania Refining Co., Butler, Pa.

† These were described in "The Tentative Draft, Minimum Requirements: Influenza Virus Vaccine, Emulsified in Mineral Oil," U. S. Dept. of H.E.W., Nat. Inst. Health, Jan. 30, 1956.