# THE EFFECT OF ACETYLCHOLINE ON THE HUMAN PULMONARY CIRCULATION UNDER NORMAL AND HYPOXIC CONDITIONS [1]

By H. W. FRITTS, Jr.,[2] P. HARRIS,[3] R. H. CLAUSS, J. E. ODELL,[4] and A. COURNAND

*(From the Departments of Medicine and Surgery, Columbia University College of Physicians and Surgeons, and the Cardio-Pulmonary Laboratory of the First Medical and Chest Services, Columbia University Division, Bellevue Hospital, New York, N. Y.)*

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It is generally accepted that the tone of the peripheral arterioles plays an important part in regulating the systemic blood pressure in man. Whether the small vessels of the lungs exercise similar control over the pressure in the pulmonary artery is not so certain. This uncertainty has stemmed largely from the fact that the human pulmonary vessels have exhibited an erratic response to many vasoactive drugs (1–15). As a consequence, most physiologists have concluded either that the pulmonary vessels are incapable of intrinsic changes in tone, or that the effect of such changes, if they occur, is less important in determining the pulmonary arterial pressure than is the effect of mechanical factors alone.

There is, however, considerable evidence to suggest that acute hypoxia increases the pulmonary vascular tone (16–20). This stimulus raises the pulmonary arterial pressure by a greater amount than might be expected to result from the increase in blood flow which occurs. Moreover, the pulmonary wedge and systemic pressures are not altered (19), and there is no consistent variation in the central blood volume (19, 21). From these observations it may be inferred that hypoxia constricts the vessels of the lungs.

Active dilatation of these vessels has not been so adequately demonstrated. Most drugs which lower the pulmonary arterial pressure have a concomitant action on the systemic pressure, and it is difficult to determine whether the changes in the pulmonary circulation represent a primary or a secondary effect. However, one of us (P.H.) observed that a single dose of acetylcholine can lower the pulmonary arterial pressure without affecting the pressure in the systemic vessels (22, 23). The response was transient, and was found only in patients with a moderate degree of pulmonary hypertension.

Since it seemed possible that this fall in pressure occurred as a result of active vasodilatation, the present project was undertaken with two objectives in view: 1) to investigate the effect of a continuous infusion of acetylcholine into the pulmonary artery of normal subjects, and 2) to inquire whether the action of the drug was enhanced when the pulmonary arterial pressure in these same subjects had been raised by making them hypoxic.

## METHODS

Each subject was studied in the unanesthetized basal state. Respiratory studies were carried out on a previous day to acquaint the subject with the laboratory personnel and to accustom him into the effects of breathing a low oxygen mixture.

Catheterization of the pulmonary artery was accomplished in the usual way (24, 25). The position of the catheter was adjusted so that the tip lay just beyond the pulmonic valve. In those subjects in whom the wedged pressure was also measured, a special double-lumen catheter was used which allowed the tip to be wedged while the proximal lumen opened into the main pulmonary artery. With the catheter in place, a cannula was introduced into the right brachial artery. The subject was then allowed to rest quietly for 20 minutes before observations were begun.

Each of the subjects breathed through a mouthpiece for two periods of 20 minutes, separated by a rest of 15 minutes. During one period 21 per cent oxygen was administered, and during the other, a mixture of 12 per

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[2] Fellow of the New York Heart Association.

[3] Fellow of the Nuffield Foundation.

[4] Postdoctoral Research Fellow, United States Public Health Service.

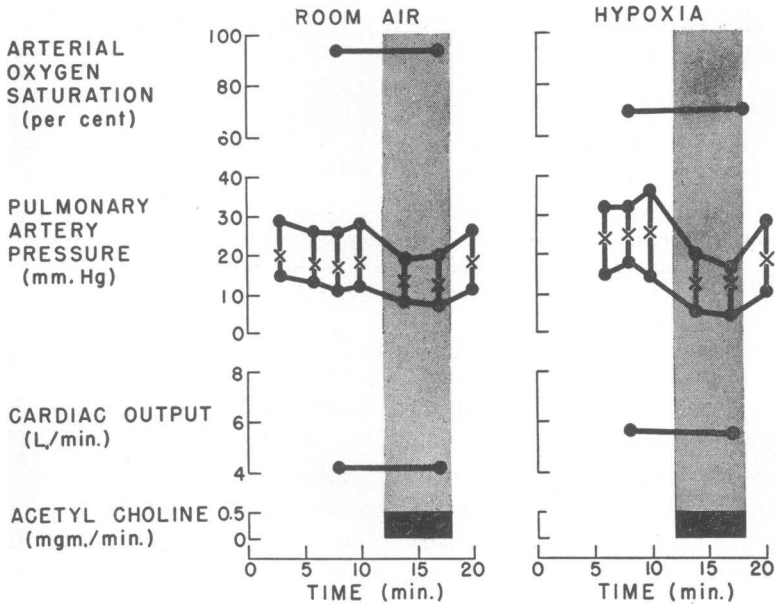


FIG. 1. TIME-COURSE OF CHANGES IN ARTERIAL OXYGEN SATURATION, PULMONARY ARTERIAL PRESSURE, AND CARDIAC OUTPUT IN SUBJECT A.B.

cent oxygen in nitrogen. In alternate patients this sequence was reversed.

Measurements of pulmonary and brachial arterial pressures, and, in three cases, pulmonary wedge pressures, were recorded at the third, sixth, eighth, and tenth minutes of each breathing period. Between the eighth and tenth minutes, a measurement of cardiac output was also made. From the eleventh minute onward, acetylcholine was infused into the main pulmonary artery at the rate of 0.5 mg. per minute. A dilution of 100 mg. acetylcholine per 100 ml. saline was used in order that the volume rate at which saline entered the pulmonary artery during the administration of the drug would equal the rate at which saline was infused during the first 10 minutes to maintain the patency of the catheter. Hence, the possibility that the saline *per se* had an effect was eliminated.

TABLE I

*Physical characteristics of the subjects studied*

| Subject | Sex | Age | Weight | Body surface area |
|---|---|---|---|---|
| | | | *Kg.* | *M.²* |
| | | Group A | | |
| A. C. | F | 30 | 72.5 | 1.78 |
| A. B. | F | 32 | 46.6 | 1.46 |
| J. M. | M | 33 | 45.0 | 1.46 |
| C. M. | M | 33 | 77.0 | 1.99 |
| J. P. | M | 30 | 77.5 | 1.97 |
| W. vP. | M | 43 | 64.8 | 1.79 |
| H. R. | F | 29 | 61.6 | 1.70 |
| R. J. | M | 32 | 70.0 | 1.81 |
| | | Group B | | |
| J. A. | M | 59 | 64.0 | 1.78 |
| W. P. | F | 33 | 65.8 | 1.75 |
| J. D. | M | 39 | 73.0 | 1.84 |
| J. G. | M | 25 | 66.2 | 1.80 |
| P. M. | M | 35 | 51.6 | 1.60 |

Pressures were recorded at the second and fourth minutes after the start of the infusion, and the cardiac output was remeasured immediately after this second pressure had been obtained. This time-sequence, which was rigidly adhered to in all studies, is illustrated in Figure 1.

The pressures were recorded with a multi-channel cathode ray oscillographic recorder, using Statham gauges as pressure transducers. Average systolic and diastolic pressures were calculated over at least two respiratory cycles, and mean pressures were obtained by planimetric integration.

While the subject breathed 21 per cent oxygen, tne cardiac output was estimated by the Fick principle. The oxygen and carbon dioxide contents of the arterial and mixed venous blood samples were determined directly, using the method of Van Slyke. Gas samples were analyzed using a Scholander microanalyzer.

Under hypoxia, the cardiac output was measured by the Stewart-Hamilton dilution principle (26). This method was thought to be preferable to the direct Fick since insufficient time was allowed for the establishment of a steady state of the pulmonary gas exchange (18). The technique entailed injecting 3 ml. of Evans blue dye through the catheter into the main pulmonary artery. A dilution curve was inscribed by sampling blood from the brachial artery through a recording densitometer (27). The densitometer was calibrated by using the pooled-sample method of McNeely and Gravallese (28).

Dye concentrations in serum were read in a Beckman Model DU spectrophotometer.

The "central blood volume" was calculated by the method of Hamilton, Moore, Kinsman, and Spurling (26). This calculated value presumably included the volume of blood in the pulmonary vessels, in the chambers of the left heart, and in those segments of the systemic arterial tree which lay at the same temporal distance from the root of the aorta as the right brachial artery.

*Subjects.* The physical characteristics of the subjects are listed in Table I. Each was a convalescent patient who was believed to have a normal pulmonary circulation. The eight subjects in Group A were studied while breathing both 21 and 12 per cent oxygen, while those in Group B were studied while breathing 21 per cent oxygen alone.

Subjects J. P. and W. vP. had had pneumonia, but had been asymptomatic for two weeks prior to the day the study was performed. In both, the abnormal X-ray shadows in the lungs had disappeared. Subject J. A. had been admitted to the hospital for treatment of nontuberculous empyema, but had been symptom-free for a period of six months. Subject R. J. exhibited a moderative degree of anemia.

## RESULTS

The data presented in Tables II and III indicate that while 21 per cent oxygen was breathed the results recorded in Groups A and B were similar. In the interest of simplifying the pre-

TABLE II

*Effect of acetylcholine on gas exchange and blood gas composition while subjects breathed 21 per cent oxygen* *

| Subject | Infusion of acetylcholine | $\dot{V}_E$ | $\dot{V}_{O_2}$ | $R_E$ | $CAP_{O_2}$ | $Ca_{O_2}$ | $(Ca_{O_2}-C\bar{v}_{O_2})$ |
|---|---|---|---|---|---|---|---|
| | | *L./min./M.²* | *ml./min./M.²* | | *ml./100 ml.* | *ml./100 ml.* | *ml./100 ml.* |
| | | | Group A | | | | |
| A. C. | Before | 4.02 | 134 | 0.80 | 15.9 | 15.2 | 4.0 |
| | During | 4.22 | 135 | 0.76 | 15.7 | 14.9 | 4.1 |
| A. B. | Before | 5.48 | 103 | 0.95 | 15.0 | 13.5 | 3.6 |
| | During | 4.93 | 102 | 0.93 | 15.0 | 13.5 | 3.6 |
| J. M. | Before | 4.60 | 138 | 0.92 | 19.2 | 18.1 | 3.7 |
| | During | 4.51 | 131 | 0.96 | 19.2 | 17.8 | 3.4 |
| C. M. | Before | 3.45 | 153 | 0.76 | 20.8 | 19.5 | 3.7 |
| | During | 3.67 | 148 | 0.83 | 20.8 | 19.4 | 3.6 |
| J. P. | Before | 4.42 | 161 | 0.78 | 15.9 | 15.0 | 3.9 |
| | During | 4.78 | 164 | 0.78 | 15.9 | 15.2 | 4.0 |
| W. vP. | Before | 6.29 | 147 | 0.76 | 16.7 | 15.4 | 4.3 |
| | During | 6.06 | 138 | 0.77 | 16.7 | 15.7 | 4.8 |
| H. R. | Before | 4.20 | 121 | 0.63 | 14.3 | 13.1 | 3.6 |
| | During | 4.37 | 142 | 0.70 | 14.3 | 13.1 | 3.6 |
| R. J. | Before | 4.49 | 138 | 0.81 | 12.5 | 11.7 | 2.8 |
| | During | 5.19 | 160 | 0.81 | 12.7 | 11.8 | 3.0 |
| | | | Group B | | | | |
| J. A. | Before | 3.91 | 117 | 0.80 | 16.3 | 15.6 | 3.9 |
| | During | 4.60 | 129 | 0.83 | 16.1 | 15.3 | 3.6 |
| W. P. | Before | 3.38 | 97 | 0.80 | 14.8 | 13.8 | 3.5 |
| | During | 3.23 | 93 | 0.82 | 14.6 | 13.7 | 3.4 |
| J. D. | Before | 4.16 | 122 | 0.82 | 19.3 | 18.0 | 4.2 |
| | During | 4.23 | 122 | 0.81 | 19.2 | 17.7 | 4.1 |
| J. G. | Before | 3.73 | 122 | 0.85 | 22.4 | 20.8 | 4.2 |
| | During | 3.97 | 146 | 0.74 | 22.6 | 21.0 | 4.4 |
| P. M. | Before | 4.26 | 134 | 0.87 | 16.8 | 15.3 | 3.7 |
| | During | 4.08 | 131 | 0.79 | 16.6 | 15.2 | 4.0 |

* The symbols used in Tables II, III, and IV are defined as follows (63): $\dot{V}_E$, volume of gas expired per minute (BTPS). $\dot{V}_{O_2}$, volume of oxygen taken up per minute (STPD). $R_E$, respiratory exchange ratio. $F_{I_{O_2}}$, fraction of oxygen in the inspired air. $Sa_{O_2}$, arterial blood oxygen saturation. $CAP_{O_2}$, arterial blood oxygen capacity. $Ca_{O_2}$, arterial blood oxygen content. $C\bar{v}_{O_2}$, mixed venous blood oxygen content.

TABLE III

*Effect of acetylcholine on arterial oxygen saturation, heart rate, cardiac index, central blood volume and pressures in the pulmonary and systemic circulations while subject breathed 21 and 12 per cent oxygen*

| Subject | $F_{I_{O_2}}$ | Infusion of acetylcholine | $Sa_{O_2}$ | Heart rate | Cardiac index | Central blood volume | Pulmonary arterial pressure* | | | Wedge pressure | Brachial arterial pressure* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | % | | *L./min./M.²* | *L./M.²* | *mm. Hg* | | | *mm. Hg* | *mm. Hg* | | |
| | | | | | Group A | | S. | D. | M. | | S. | D. | M. |
| A. C. | 0.21 | Before | 98 | 90 | 3.3 | | 18 | 8 | 12 | | 146 | 95 | 116 |
| | | During | 98 | 89 | 3.3 | | 16 | 7 | 11 | | 140 | 91 | 115 |
| | 0.12 | Before | 71 | 108 | 4.6 | 0.637 | 36 | 16 | 24 | | 131 | 87 | 108 |
| | | During | 67 | 104 | 4.8 | 0.676 | 22 | 10 | 15 | | 134 | 91 | 111 |
| A. B. | 0.21 | Before | 93 | 61 | 2.9 | | 27 | 12 | 18 | | 108 | 65 | 83 |
| | | During | 93 | 57 | 2.8 | | 20 | 8 | 13 | | 103 | 65 | 82 |
| | 0.12 | Before | 69 | 71 | 3.8 | 0.665 | 34 | 16 | 25 | | 104 | 70 | 82 |
| | | During | 70 | 78 | 3.8 | 0.640 | 18 | 5 | 12 | | 100 | 59 | 77 |
| J. M. | 0.21 | Before | 96 | 72 | 3.7 | | 21 | 6 | 11 | 9 | 123 | 75 | 94 |
| | | During | 95 | 72 | 3.8 | | 19 | 6 | 11 | 10 | 121 | 71 | 94 |
| | 0.12 | Before | 75 | 89 | 4.7 | 0.740 | 24 | 7 | 16 | 10 | 120 | 68 | 89 |
| | | During | 68 | 90 | 6.6 | 1.200 | 24 | 7 | 15 | 10 | 124 | 68 | 88 |
| C. M. | 0.21 | Before | 95 | 81 | 4.1 | | 25 | 12 | 18 | 9 | 145 | 85 | 113 |
| | | During | 95 | 76 | 4.1 | | 23 | 11 | 16 | 9 | 141 | 85 | 108 |
| | 0.12 | Before | 70 | 93 | 5.6 | 0.837 | 33 | 15 | 23 | 6 | 145 | 87 | 110 |
| | | During | 64 | 91 | 5.5 | 0.802 | 26 | 12 | 20 | 7 | 146 | 87 | 111 |
| J. P. | 0.21 | Before | 97 | 78 | 4.1 | | 13 | 5 | 10 | | 134 | 79 | 98 |
| | | During | 98 | 80 | 4.1 | | 12 | 5 | 9 | | 137 | 83 | 103 |
| | 0.12 | Before | | 85 | 6.2 | 0.857 | 18 | 8 | 12 | | 146 | 83 | 103 |
| | | During | 78 | 82 | 6.8 | 0.854 | 14 | 7 | 11 | | 142 | 79 | 100 |
| W. vP. | 0.21 | Before | 95 | 73 | 3.4 | | 30 | 13 | 20 | | 150 | 94 | 113 |
| | | During | 96 | 71 | 2.9 | | 25 | 11 | 17 | | 150 | 92 | 117 |
| | 0.12 | Before | | 86 | 5.9 | 0.855 | 36 | 15 | 24 | | 149 | 91 | 117 |
| | | During | 76 | 81 | 6.5 | 0.989 | 33 | 13 | 22 | | 146 | 85 | 112 |
| H. R. | 0.21 | Before | 94 | 81 | 3.4 | | 20 | 9 | 14 | 7 | 112 | 72 | 93 |
| | | During | 94 | 90 | 3.9 | | 19 | 8 | 13 | 7 | 116 | 75 | 93 |
| | 0.12 | Before | 77 | 104 | 4.9 | 0.608 | 24 | 12 | 17 | 5 | 113 | 75 | 89 |
| | | During | 81 | 113 | 5.3 | 0.644 | 21 | 11 | 14 | 5 | 111 | 74 | 88 |
| R. J. | 0.21 | Before | 96 | 76 | 5.0 | | 26 | 13 | 19 | | 130 | 84 | 100 |
| | | During | 96 | 75 | 5.3 | | 25 | 12 | 17 | | 133 | 85 | 102 |
| | 0.12 | Before | 82 | 83 | 4.6 | 1.159 | 29 | 15 | 21 | | 123 | 80 | 95 |
| | | During | 79 | 80 | 5.8 | 0.910 | 23 | 11 | 17 | | 127 | 81 | 97 |
| | | | | | Group B | | | | | | | | |
| J. A. | 0.21 | Before | 98 | 70 | 3.0 | | 28 | 12 | 18 | | 125 | 78 | 100 |
| | | During | 97 | 73 | 3.6 | | 30 | 13 | 19 | | 131 | 86 | 104 |
| W. P. | 0.21 | Before | 96 | 76 | 2.8 | | 15 | 5 | 11 | | 123 | 85 | 103 |
| | | During | 96 | 84 | 2.7 | | 14 | 4 | 10 | | 119 | 82 | 99 |
| J. D. | 0.21 | Before | 95 | 78 | 2.9 | | 15 | 6 | 10 | | 109 | 71 | 91 |
| | | During | 94 | 76 | 2.9 | | 6 | 3 | 4 | | 115 | 75 | 91 |
| J. G. | 0.21 | Before | 94 | 97 | 2.8 | | 19 | 9 | 14 | | 126 | 77 | 95 |
| | | During | 95 | 101 | 3.3 | | 19 | 10 | 14 | | 128 | 81 | 97 |
| P. M. | 0.21 | Before | 93 | 86 | 3.6 | | 27 | 11 | 18 | | 153 | 95 | 119 |
| | | During | 94 | 88 | 3.3 | | 24 | 10 | 17 | | 154 | 95 | 122 |

* S., systolic; D., diastolic; M., mean.

sentation of the data, therefore, only the results obtained in the eight subjects in Group A will be discussed. As previously noted, these eight subjects were studied while breathing both 21 and 12 per cent oxygen. The effects of acetylcholine at both levels of oxygenation are summarized in Table IV.

TABLE IV

*Summary of the average values obtained in the eight subjects in group A* *

| | $F_{IO_2}$. | Before infusion | During infusion |
|---|---|---|---|
| Ventilation (L./min./M.²) | 0.21 | 4.62 | 4.72 |
| | 0.12 | | |
| Arterial oxygen saturation (%) | 0.21 | 96 | 96 |
| | 0.12 | 74† | 72† |
| Heart rate | 0.21 | 77 | 76 |
| | 0.12 | 90 | 90 |
| Cardiac index (L./min./M.²) | 0.21 | 3.74 | 3.78 |
| | 0.12 | 5.04 | 5.64 |
| "Central blood volume" (L./M.²) | 0.21 | | |
| | 0.12 | 0.795 | 0.839 |
| Pulmonary arterial pressure (mm. Hg) | 0.21 | 23/10 (15) | 20/9 (13) |
| | 0.12 | 29/13 (20) | 23/10 (16) |
| Pulmonary wedge pressure (mm. Hg)‡ | 0.21 | 8 | 9 |
| | 0.12 | 7 | 7 |
| Brachial arterial pressure (mm. Hg) | 0.21 | 131/81 (101) | 130/81 (101) |
| | 0.12 | 129/80 (99) | 129/78 (98) |

* With the exception of the Ventilation, Cardiac index, and "Central blood volume," the average values have been rounded off to the nearest whole number.
† Measured in six subjects.
‡ Measured in three subjects.

### *A. Pressure in the pulmonary artery*

Since several factors were operative which might alter the pulmonary arterial pressure, the experimental protocol was set up according to the factorial design devised by Fisher (29). The data, therefore, have been analyzed by the analysis of variance. This approach allows the questions posed earlier to be answered; namely, 1) does acetylcholine affect the pulmonary arterial pressure, and 2) is the effect more pronounced during hypoxia?

The results of the analyses are included in the Appendix. They indicate that the fall in the pulmonary arterial mean pressure produced by acetylcholine would have arisen by chance less frequently than 1 in 20 times ($F = 12.18$, $0.05 > p > 0.01$). They further indicate that the heightened response during hypoxia would also have arisen by chance less frequently than 1 in 20 times ($F = 7.15$, $0.05 > p > 0.01$). Separate analyses of these data by the simpler "t" test showed effects of the same order of magnitude, both when breathing ambient air ($t = 2.9396$, DF (degrees of freedom) $=7$, $0.05 > p > 0.02$) and under hypoxia ($t = 3.3304$, $DF = 7$, $0.02 > p > 0.01$). In all these analyses, the two measurements made immediately before the infusion was

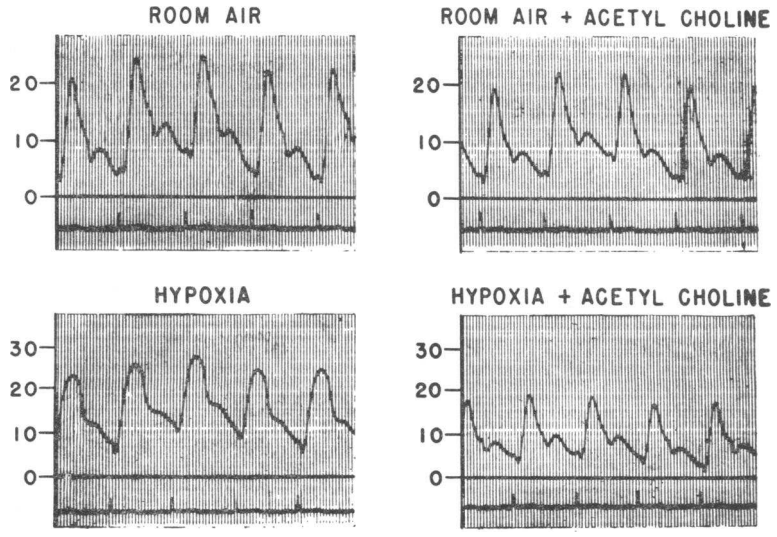


FIG. 2. INFLUENCE OF HYPOXIA AND ACETYLCHOLINE ON THE PRESSURE PULSE RECORDED FROM THE PULMONARY ARTERY

begun were compared with the two made after the infusion had been started.

As can be seen in Table III, the effect of acetylcholine on the systolic and diastolic pressures was similar to the effect on the mean pressure. Of particular interest is the fall in systolic pressure produced by the drug when the subjects breathed ambient air. This fall would have arisen by chance less frequently than 1 in 100 times ($F = 13.06$, $p < 0.01$).

It is noteworthy that in three subjects acetylcholine altered the contour of the pressure pulse recorded from the pulmonary artery. An example is shown in Figure 2. It can be seen that hypoxia produced a rounding of the systolic peak such as is seen in vasoconstriction. With the administration of acetylcholine, the characteristics of the pulse contour returned to those observed before the application of the hypoxic stimulus.

### *B. Pulmonary wedge pressure*

Because the experimental protocol necessitated measuring several variables within a limited period of time, it was not possible to secure records of wedge pressure in all of the studies. However, in three subjects simultaneous tracings of wedge and pulmonary arterial pressures were recorded by using the double-lumen catheter described under Methods. In none of these subjects was the wedge pressure affected by either hypoxia or acetylcholine.

### *C. Cardiac output*

While breathing 21 per cent oxygen, the average cardiac index was 3.74 L. per minute per M.$^2$ before the drug was administered, and 3.78 L. per minute per M.$^2$ during the period of the infusion. An analysis of these data indicated that the difference between these mean values could easily have arisen by chance ($t = 0.3631$, $DF = 7$, $p > 0.5$).

After 12 minutes of hypoxia, the average cardiac index had increased to 5.04 L. per minute per M.$^2$. During the infusion, the average was 5.63 L. per minute per M.$^2$. This difference between the cardiac indices measured before and during the infusion would have arisen by chance less frequently than 1 in 20 times ($t = 2.5432$, $DF = 7$, $0.05 > p > 0.02$). In every subject the cardiac output either remained unchanged or increased slightly while the drug was being infused. In only two subjects (J. M. and R. J.) did this change in output exceed 10 per cent, the figure usually given as the reproducibility of this measurement under basal conditions.

Thus, the fall in pulmonary arterial pressure during the administration of acetylcholine could not be ascribed to a reduction in pulmonary blood flow.

### *D. Heart rate*

There was no evidence that acetylcholine affected the heart rate. While the subjects breathed 21 per cent oxygen the average rate was 76.5, and during the infusion of acetylcholine, 76.3. Although with hypoxia the average rate increased to 89.9, an identical value was recorded during the infusion.

### *E. Pressure in the brachial artery*

As can be seen in Table IV, the average systolic, diastolic, and mean pressures in the brachial artery were not altered by either hypoxia or acetylcholine.

### *F. Central blood volume*

The effect of acetylcholine on the central blood volume was studied only during hypoxia. There was no consistent pattern of change. The average value before infusion was 0.795 L. per M.$^2$, and during the infusion 0.839 L. per M.$^2$. This difference could have arisen by chance ($t = 0.6300$, $DF = 7$, $p > 0.5$).

It should be emphasized that this is a crude measurement, and that these results do not rule out the possibility that small changes in volume occurred. For instance, in a previous study, two successive measurements of the central blood volume were made under basal conditions in eight normal subjects (21). It was found that the standard deviation of the differences between each pair of measurements was 9 per cent of the mean. It was also established that acute hypoxia did not produce alterations which lay beyond this range.

*G. Arterial oxygen saturation*

Acetylcholine did not change the arterial oxygen saturation while the subjects breathed 21 per cent oxygen. The maximum difference observed in any single subject was only 1 per cent. The average values of saturation before and during the infusion were 95.5 and 95.6 per cent, respectively.

At the end of 12 minutes of hypoxia, the average saturation of the six subjects in whom it was measured had fallen to 74 per cent. During the period of infusion the average saturation in these same subjects was 71.5 per cent. Thus, the fall in the pulmonary arterial pressure during the infusion could not be ascribed to a lessening of the hypoxic stimulus.

*H. Ventilation*

The ventilation was measured only while 21 per cent oxygen was breathed. Acetylcholine had no apparent effect. Before the drug was administered the avérage ventilation was 4.62 L. per minute per M.$^2$, and during the infusion 4.72 L. per minute per M.$^2$.

## DISCUSSION

*Effect of acetylcholine on the pulmonary vessels*

The recognition of vasomotor activity depends on the interpretation of alterations in resistance. This resistance is calculated by dividing the pressure drop across a segment of the circulation by the volume rate at which it is perfused with blood. But, in addition to the vascular tonus, this ratio depends on several other factors, notably the rate of flow, the pressures at the ends of the system, and the composition of the blood itself (30–36). Hence, any change in resistance must be interpreted with caution. On the one hand, it may indicate vasomotor activity. On the other, it may simply reflect a variation in one of the associated factors.

In the pulmonary circulation the situation is even further complicated by the variability of the pressure outside the vessels and by the fact that the volume of blood in the lungs can be altered by systemic vasocontriction or dilatation. For all of these reasons it would seem to be impossible to estimate changes in vasomotor tone in any exact way. However, one can be reasonably certain that vasodilatation has occurred if the pulmonary arterial pressure falls in the face of the following conditions: 1) a constant or increased cardiac output; 2) a constant pressure in the left atrium; 3) an unchanged extravascular pressure within the lungs and thorax; and 4) a constant heart rate, systemic pressure, and pulmonary blood volume.

Using these criteria, an examination of our data reveals the following points. Acetylcholine lowered the pulmonary arterial pressure while the cardiac output either remained constant or increased. In the three subjects in whom an assessment of left atrial pressure was obtained by using the wedged catheter technique, no change in this pressure was observed. Although the extravascular pressure within the lungs and thorax was not measured, there was no evidence that any change took place; for instance, the minute volume of ventilation and the respiratory frequency remained constant, and no respiratory symptoms developed. Finally, the heart rate and systemic blood pressure were not altered, and the central blood volume, which includes the volume of blood in the pulmonary vessels, showed no consistent variation. These observations, which are in conformity with limited results previously reported (37, 38), suggest that acetylcholine dilated the vessels of the lungs.

*The mechanism whereby the effect of acetylcholine is mediated*

On the basis of these data, it may be presumed that the drug in some way acted on the muscle fibers of the medial coats of the small branches of the pulmonary vascular tree. Theoretically, it might have affected these fibers directly, reaching them either by diffusion through the vessel wall or via the vasa vasorum from the bronchial circulation. The possibility that diffusion through the wall occurred seems plausible, since it has been demonstrated that acetylcholine can alter the tone of vascular rings *in vitro* (39, 40).

If instead the drug reached the pulmonary vessels by recirculation through the bronchial arteries, it might have been expected to have caused vasodilatation elsewhere in the systemic circula-

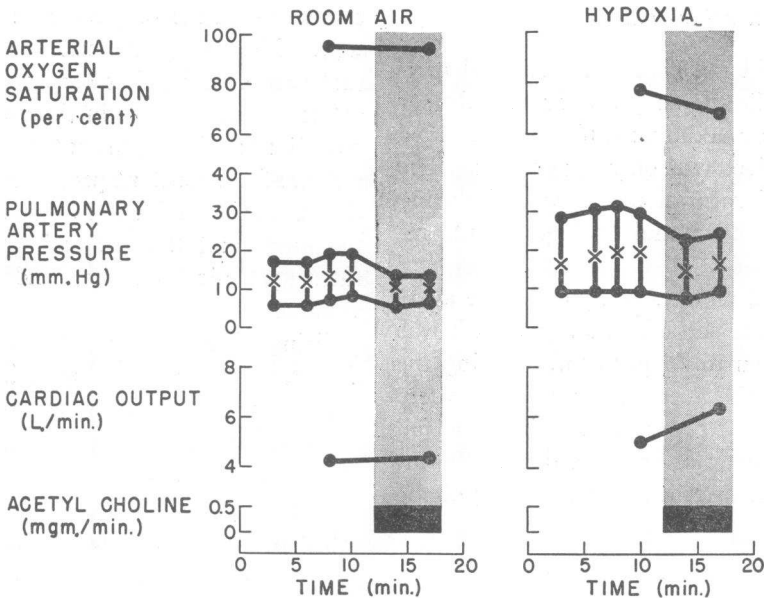


FIG. 3. TIME-COURSE OF CHANGES IN ARTERIAL OXYGEN SATURATION, PULMONARY ARTERIAL PRESSURE AND CARDIAC OUTPUT IN SUBJECT E.B. WHO HAD HAD A TOTAL SYMPATHECTOMY

tion or to have produced bronchoconstriction. No such effects were evident.

Alternatively, the action of the drug may have been transmitted by means of nervous pathways. One such possibility can be ruled out on the basis of a study performed on a subject who had had a complete surgical sympathectomy. The results are shown in Figure 3. Fishman, Himmelstein, Fritts, Lahoz, and Cournand (20) have previously reported that hypoxia can produce pulmonary hypertension in a patient in whom the cervical and upper four sympathetic ganglia have been resected. The present results suggest that both the effect of hypoxia and of acetylcholine can be mediated with all of the sympathetic ganglia removed.

*Effect of hypoxia on the response to acetylcholine*

The influence of acetylcholine was in general more apparent during hypoxia than when the same subjects breathed room air. It is noteworthy, also, that this increased action of acetylcholine under hypoxia was most striking in those patients in whom the effect of hypoxia on the pulmonary circulation was greatest. Hence, it might be suspected that hypoxia and acetylcholine are in some way synergistic.

But in an earlier study one of us observed that a single dose of acetylcholine had the same marked effect in patients who had developed pulmonary hypertension as a consequence of heart disease (22, 23). The effect was transient, and was not evident in patients in whom the pulmonary arterial pressure was normal. To explain these observations it was suggested that the response depends on the preexisting vascular tone and the thickness of the media of the small branches of the pulmonary artery.

Since there is considerable evidence to indicate that hypoxia constricts the vessels of the lungs (16–20, 41, 42), it seems likely that an augmented tone is the common factor in the previous and the present results. There does not appear to be any evidence that hypoxia *per se* potentiates the effect of acetylcholine.

*Results reported by others*

Finally, the results of the present study must be examined in the light of those which have been reported by others. Most of the experiments have

been carried out on laboratory animals (43–56). A review of these papers indicates that no consistent pattern of response was obtained.

In man, intravenous injections of acetylcholine have been shown to cause vasodilatation in the systemic circulation. Villaret and Justin-Besançon (57), Ellis and Weiss (58, 59), and Carmichael and Fraser (60) all observed that intravenous doses of the drug produced either a fall in blood pressure or a flushing of the skin. Ellis and Weiss (58) fixed the minimal effective infusion rate as lying between 50 and 60 mg. per minute. It should be pointed out that this is from 80 to 120 times greater than the dosage used in the present study. It is believed, therefore, that with the infusion of 0.5 mg. per minute the effect of the drug was largely dissipated before it reached the left side of the heart and the systemic circulation.

This belief is supported by the results of Duff, Greenfield, Shepherd, and Thompson (61) who investigated the rapidity with which cholinesterase inactivates acetylcholine in the bloodstream. Using a plethysmograph to measure changes in blood flow, they demonstrated that as little as 0.00025 mg. acetylcholine would augment the flow through the forearm when injected into the brachial artery. The dose had to be increased about one thousand fold in order to augment the blood flow through the hand. They attributed this phenomenon to the inactivation of the drug by the enzyme. Moreover, they found that when acetylcholine was incubated with blood for 10 seconds before the injection, the potency of the dose was diminished by more than 99.7 per cent of its original strength. These results confirm the early observations of Tiffeneau and Beauvallet (62), who demonstrated that, once introduced into the cardiovascular system, the effect of acetylcholine is rapidly neutralized.

### *Conclusions*

It is tempting to say that our results indicate that acetylcholine, a naturally occurring substance, plays a role in controlling the pulmonary arterial pressure in normal man. But this conclusion is not justified. Pharmacologic, not physiologic, doses have been used and marked responses have been observed only when the pulmonary arterial pressure was elevated. Thus it may be that acetylcholine plays a part in regulating the pulmonary circulation in disease states in which pulmonary hypertension exists.

From a practical standpoint, the data suggest that acetylcholine or a related substance of longer action might be useful in the therapy of patients with acute or chronic pulmonary hypertension. The drug might also provide information regarding the status of the pulmonary vessels of patients in whom pulmonary hypertension has been present for a prolonged period of time.

## SUMMARY

Acetylcholine has been infused into the pulmonary artery of normal human subjects under normal and hypoxic conditions. The drug caused a fall in pulmonary arterial pressure which was more evident after hypoxia had produced pulmonary hypertension. The fall in pressure was not associated with a fall in cardiac output, and there was no change in the pulmonary wedge pressure, heart rate, systemic blood pressure, or central blood volume.

It is concluded that acetylcholine causes pulmonary vasodilatation. Apparently the effect is enhanced in the presence of an increased vascular tone.

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## APPENDIX

*Analysis of variance of mean pulmonary arterial pressure in the eight subjects of group A*

| Source of variance | $S(X-\bar{X})^2$ | DF | Variance |
|---|---|---|---|
| Total | 1257.4 | 63 | |
| Subjects | 630.4 | 7 | 90.06 |
| Acetylcholine | 168.9 | 1 | 168.9 |
| $F_{I_{O_2}}$ | 210.2 | 1 | 210.2 |
| Subjects × acetylcholine | 97.1 | 7 | 13.87 |
| Subjects × $F_{I_{O_2}}$ | 63.3 | 7 | 9.043 |
| Acetylcholine × $F_{I_{O_2}}$ | 39.1 | 1 | 39.1 |
| Subjects × acetylcholine × $F_{I_{O_2}}$ | 38.3 | 7 | 5.471 |
| Residuum | 10.1 | 32 | 0.3156 |

*Analysis of variance of systolic pulmonary arterial pressure in the eight subjects of group A*

| Source of variance | $S(X-\bar{X})^2$ | DF | Variance |
|---|---|---|---|
| Total | 2615.4 | 63 | |
| Subjects | 1357.5 | 7 | 193.9 |
| Acetylcholine | 337.7 | 1 | 337.7 |
| $F_{I_{O_2}}$ | 375.4 | 1 | 375.4 |
| Subjects×acetylcholine | 181.0 | 7 | 25.86 |
| Subjects×$F_{I_{O_2}}$ | 166.8 | 7 | 23.83 |
| Acetylcholine×$F_{I_{O_2}}$ | 62.0 | 1 | 62.0 |
| Subjects×acetylcholine×$F_{I_{O_2}}$ | 88.9 | 7 | 12.7 |
| Residuum | 46.1 | 32 | 1.44 |

*Analysis of variance of diastolic pulmonary arterial pressure in the eight subjects of group A*

| Source of variance | $S(X-\bar{X})^2$ | DF | Variance |
|---|---|---|---|
| Total | 709.4 | 63 | |
| Subjects | 355.4 | 7 | 50.77 |
| Acetylcholine | 85.5 | 1 | 85.5 |
| $F_{I_{O_2}}$ | 68.0 | 1 | 68.0 |
| Subjects×acetylcholine | 84.5 | 7 | 12.07 |
| Subjects×$F_{I_{O_2}}$ | 36.5 | 7 | 5.214 |
| Acetylcholine×$F_{I_{O_2}}$ | 22.6 | 1 | 22.6 |
| Subjects×acetylcholine×$F_{I_{O_2}}$ | 50.5 | 7 | 7.214 |
| Residuum | 6.4 | 32 | 0.2 |

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