mouse. Treatment was withheld until 22 hours after inoculation when 10% of the animals had died. The 90 survivors were divided at random into 3 treatment groups as outlined above.

In addition, control experiments were performed which showed that adrenal cortical extract, corn oil, penicillin and saline injections in the dosages and schedules used in the experiments had no noticeably harmful effects on uninfected mice.

Results and comments. The mortality in the 3 treatment groups is indicated in Table I for the first experiment and in Table II for the second experiment. As would be expected, the groups treated with penicillin showed a much lower mortality than the controls receiving no penicillin. No further improvement in mortality was effected by additional treatment with large doses of potent adrenal cortical extract. If these experiments are applicable to clinical situations, the results do not lend encouragement to the use of adrenal cortical extracts in the treatment of patients moribund from bacterial infections.

Summary. Mice of varying weights were inoculated intraperitoneally with large numbers of Type I pneumococci and the infections allowed to proceed until from 5 to 10% of the animals had succumbed. The survivors were divided into groups at random and treated with penicillin and penicillin plus potent adrenal cortical extract. No improvement was obtained in the therapeutic results achieved with penicillin by the additional treatment with adrenal cortical extract.

We wish to thank Dr. E. Gifford Upjohn of the Upjohn Pharmaceutical Company for generously supplying us with Lipo-Adrenal Cortex (Upjohn).

16780

Reproduction of Human Ulcerative Pulmonary Tuberculosis in Rabbits by Quantitative Natural Airborne Contagion.*

MAX B. LURIE AND SAMUEL ABRAMSON.†

From the Henry Phipps Institute, University of Pennsylvania, Philadelphia.

The purpose of this report is to outline a study in which the acquisition and development of human ulcerative pulmonary tuberculosis was closely simulated in rabbits by exposing them to the inhalation of known numbers of tubercle bacilli while breathing naturally in an experimental apparatus.

Materials and methods. An apparatus for airborne infection of rabbits, constructed at the Cornell University Medical School, was placed at our disposal by Dr. Eugene L. Opie. This was modified in accordance with the principles elaborated by Wells[1] and further adjusted by certain appliances directed toward improving its quantitative aspects.

Fig. 1 gives a schematic drawing of the instrument. Briefly, a fine suspension of largely isolated, virulent bovine type tubercle bacilli, freed from clumps larger than a red blood cell by filtration through Whatman number 5 filter paper, is atomized with a known volume of compressed air in unit time through a specially designed nozzle. The large droplets settle out quickly in the spraying flask. A uniform flow of droplet nuclei, containing tubercle bacilli, is delivered into a mixing device where it is diluted with room air. Thence the contaminated air is drawn through a 16-foot pipe into a chamber where the rabbits are exposed. This chamber is exhausted by the draft action of a hot flame at the bottom of a baffled chimney connected

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† Tuberculosis Control Division, Public Health Service, Federal Security Agency.

1 Wells, W. F., *Science*, 1940, **91**, 172.

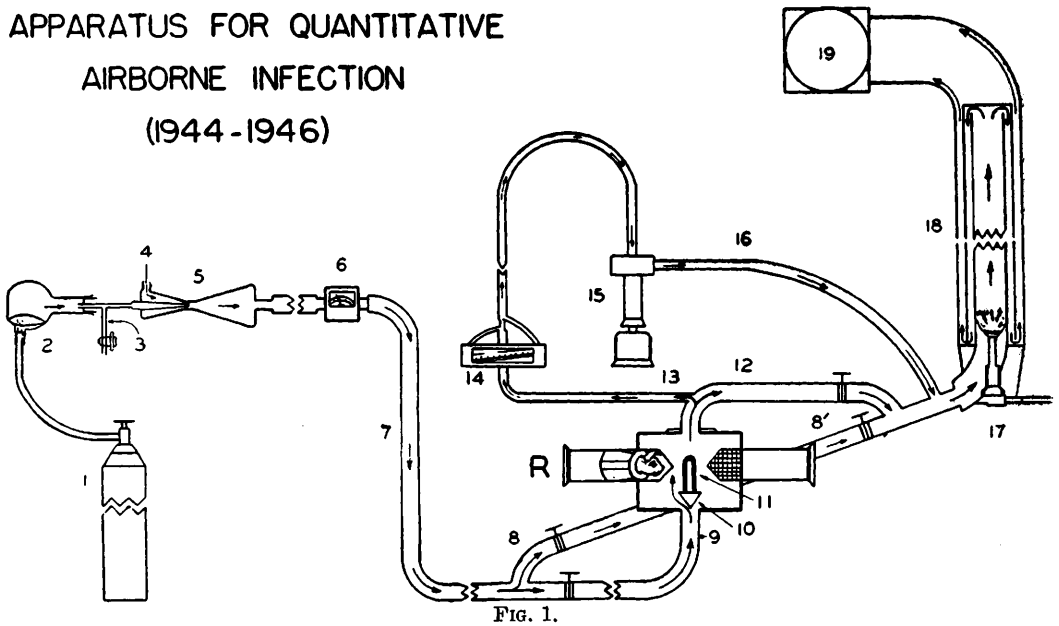

FIG. 1.

1—Compressed air tank. 2—Atomizer and spraying flask. 3—Outlet for sampling aerosol. 4—Air inflow from room. 5—Mixing device. 6—Flow-meter (liters/min.). 7—Tube which conveys infected air. 8-8'—Shunt. 9—Inlet tube to exposure chamber. 10—Spreading cone. R—Rabbit in cylinder, with head protruding into exposure chamber through rubber collar about its neck. 11—Ultraviolet lamp. 12—Outlet tube from exposure chamber. 13—Outlet for sampling exposure chamber. 14—Inclined draft gauge. 15—Wells air centrifuge. 16—Air centrifuge exhaust tube. 17—Burner. 18—Baffled incinerating chimney. 19—Exhaust fan.

to the outlet of the chamber. The incinerated air is exhausted to the outdoor atmosphere by a fan.

The concentration of tubercle bacilli in the air respired by the exposed rabbits is determined culturally[2] with the aid of a Wells air centrifuge, provided with a calibrated inclined draft gauge, which gives the exact volume of air sampled. Since the total volume of contaminated air respired by the rabbits during their period of exposure can be determined,[3] the number of bacilli to which they were exposed can be calculated.

If the bacilli in the exposure chamber are too few to be accurately determined directly, they can be estimated by ascertaining their number in the aerosol immediately after it leaves the spraying flask and before it is diluted by the inflowing room air. The dilution factor existing between the concentration of bacilli in this aerosol and that in the exposure chamber can be empirically determined by numerous preliminary calibrations. This factor depends, in part, on the volume of room air added to a unit volume of aerosol in a given time, and can be calculated from data obtained by a flowmeter which measures the rate of air flow through the system. This determination is made possible by introducing a shunt into the duct which feeds the infected air to the exposure chamber. By means of this shunt, which is provided with appropriate valves, the infected air can be made to bypass the exposure chamber in which the rabbits are placed, and in which they can breathe uninfected air while the concentration of bacilli in the aerosol is ascertained. At the desired moment, without altering the inflow of infected air and without danger to the operators, the valves can be adjusted and the contaminated air allowed to flow through the exposure chamber.

Experimental. Twenty-nine rabbits, of unknown genetic resistance to tuberculosis, in groups of 2 to 5, in 9 separate experiments were exposed to the inhalation of varying numbers of highly virulent bovine type tuber-

[2] Lurie, M. B., *J. Exp. Med.*, 1934, **60**, 163.

[3] Murphy, D. C., and Thorpe, E. S., *J. Clin. Invest.* 1931, **10**, 545.

TABLE I.
Relation Between the Number of Tubercle Bacilli Respired and the Number of Tubercles Developed.

1	2	3	4	5	6
Exp. No.	No. of tubercle bacilli per l. respired air	Rabbit No.	No. of tubercle bacilli calculated as respired	No. of primary tubercles found in both lungs	Ratio between No. of tubercle bacilli respired and No. of tubercles in lungs
1	1217	1	8,819	591	14.9
	,,	2	8,041	973	8.2
	,,	3	10,116	1,270	8.0
2	643	4	3,858	1,344	2.8
	,,	5	4,629	833	5.5
3	468	6	2,808	425	6.6
	,,	7	2,574	605	4.2
	,,	8	2,808	397	7.1
	,,	9	2,808	387	7.2
4	228	10	1,436	574	2.5
	,,	11	1,276	397	3.2
	,,	12	1,413	1,012 tubercle bacilli recovered from lungs	
5	91	13	627	59	10.6
	,,	14	664	82	8.1
	,,	15	700	38	18.4
	,,	16	664	48	13.8
	,,	17	609	104	5.8
6	87	18	522	130	4.0
	,,	19	609	59	10.3
	,,	20	600	105	5.7
7	17	21	114	5	23.0
	,,	22	122	15	8.1
8	13	23	78	18	4.3
	,,	24	78	39	2.0
	,,	25	74	16	4.6
	,,	26	75	100 tubercle bacilli recovered from lungs	
9	5	27	32	3	10.1
	,,	28	32	1	32.0
	,,	29	31	0	—
				Avg	9 ± 7

cle bacilli of the strain Ravenel grown on a modified Lowenstein medium.[2] The number of bacillary units in the air respired by the rabbits in the individual experiments ranged from 5 to over 1200 per liter, and are listed in column 2, Table I.

Given the duration of exposure of each rabbit to the known concentration of bacilli in its respired air, the total number of bacillary units to which the rabbits were exposed can be calculated by Kleiber's formula.[4] This states that animals inhale 212 cc of air per minute per kilo of body weight to the three-quarter power in order to satisfy their oxygen needs. Column 4 lists these figures.

That the number of bacilli calculated to have been inhaled by this method is not far from the actual number of microorganisms found in the lung immediately after exposure has been noted previously.[5] It is also suggested in this table. It will be seen that in

[4] Kleiber, M., *Science*, 1944, **99**, 542.

[5] Wells, W. F., and Lurie, M. B., *Am. J. Hyg.*, 1941, **34**, Sect. B, 21.

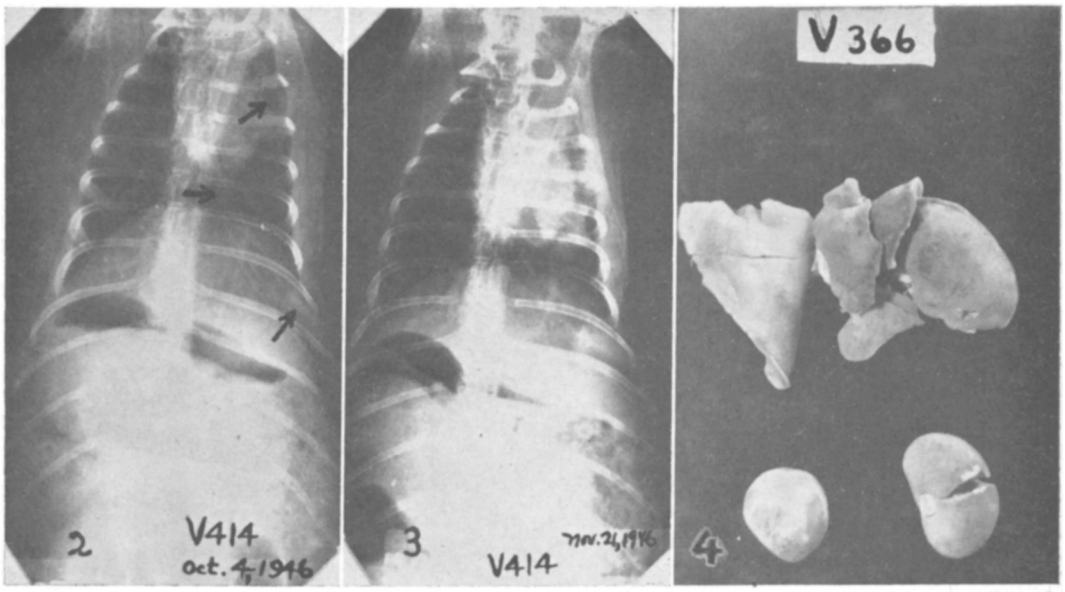

FIG. 2. Radiograph of rabbit V 414, 67 days after exposure. Three foci are visible. A walled off cavity in the third interspace and areas of consolidation in the sixth and eighth interspaces, respectively, of the left lung are indicated by arrows.

FIG. 3. Radiograph of the rabbit shown in Fig. 2, 48 days later. The progression of the disease in the left lung is evident. The right lung is normal.

FIG. 4. The lungs and kidneys of rabbit V 366 at death, 9.5 months after exposure. One of the 3 primary cavities found in the right lung is seen in the lower lobe. Infarcts of nontuberculous origin in both kidneys.

experiment 4 it was estimated that rabbit 12 had inhaled 1413 bacillary units. Actually, 1012 tubercle bacilli were recovered from its lungs immediately after exposure, as recorded in column 5. Again, in experiment 8, it was calculated that rabbit 26 had inhaled 75 bacilli. Actually, column 5 shows that 100 organisms were cultured from its lungs. Similarly close checks between the bacilli calculated as inhaled and the number recovered from the lungs have been observed since on many occasions.

Five to 6 weeks after exposure the rabbits were killed and the number of primary tubercles present in their lungs was carefully determined by excising each individual focus in both lungs. In those instances where the tubercles were too numerous for direct counting, these were estimated as follows: After removing the bronchi at their junction with the pulmonary parenchyma, both lungs were weighed. One lobe from each lung was also accurately weighed and the number of tubercles in these directly counted by enucleation of each nodule. By simple ratio the number of tubercles present in both lungs was calculated. This procedure was justified because, in these instances, the tubercles were uniformly distributed throughout both lungs.

It will be noted in columns 4 and 5 that there is a general correlation between the number of primary tubercles found in the lungs and the number of bacillary units estimated to have been inhaled. The larger the number inhaled the larger the number of primary pulmonary foci. However, there is no constant ratio between the number of bacilli required to generate a single tubercle in the different experiments, nor even in different rabbits of the same experiment inhaling the same infected air. This ratio is listed in column 6, and ranged from 2 to 32 bacillary units per tubercle in the 9 experiments. In one and the same experiment this ratio was often 3 times greater in one animal than in another. The average ratio in all the 26 rabbits was 9 ± 7 bacillary units per tubercle generated. Whether this ratio depends on the native resistance of rabbits or on other factors has not yet been determined.

TABLE II.
Fate of Rabbits of Race III, Sensitized with Heat-killed Tubercle Bacilli and Exposed 5.5 Months Later to the Inhalation of About 50 Virulent Bovine Tubercle Bacilli.

Rabbit No.	Survival after exposure, mo.	Type of tuberculosis
V 369*	Still living, 26	No evidence of tuberculosis.
U 784	Killed, 4.7	No tuberculosis.
V 366	9.5	3 small, well walled off cavities in right lung. No tuberculosis elsewhere, including hilum nodes. Large infarcts in both kidneys.
V 30	10.1	1 large cavity with limited bronchogenic spread in upper lobe of each lung. Hilum nodes normal. Ulcerative tuberculosis of larynx. Single miliary tubercle in one kidney.
V 414	7.3	Unilateral ulcerative pulmonary tuberculosis with slight, contralateral lesions. Hilum nodes normal. Miliary tubercles in each kidney. Ulcerative laryngeal tuberculosis. One large tuberculous pleural nodule. Tuberculosis of one wrist joint.
V 267	6.6	Completely excavated tuberculosis of all lobes of right lung, including the azygous lobe. Consolidation of upper lobe of left lung and bronchogenic spread to lower lobe of same lung. Hilum nodes normal. Few miliary tubercles in one kidney.

* This rabbit was not sensitized with heat-killed tubercle bacilli before exposure.

Using the above observations as a basis, a group of 5 highly inbred rabbits of Race III, obtained from Dr. Paul B. Sawin of the Roscoe B. Jackson Memorial Laboratory, were given 6 consecutive, weekly intracutaneous injections of heat-killed bovine type tubercle bacilli, totaling 7 mg per rabbit. Five and one-half months after the last injection of heat-killed tubercle bacilli, these 5 vaccinated rabbits together with a sixth unvaccinated animal of the same race were exposed simultaneously in the apparatus for 10 minutes to droplet nuclei of virulent bovine type tubercle bacilli of the Ravenel strain derived from a culture on glycerol agar. There were 10 bacillary units per liter in the air respired by these rabbits. On the basis of the above noted Kleiber formula it was estimated that each rabbit inhaled about 5 liters of this infected air, or about 50 bacilli. Two months after exposure some of the rabbits showed radiographic evidence of sharply defined pulmonary lesions, some with cavity formation (Fig. 2); 2-3 such foci were found in these rabbits at this time. It must be noted that preliminary studies on this inbred race of rabbits had shown that they were of high genetic resistance to tuberculosis.

Table II details the results of this experiment. It will be noted that the unvaccinated rabbit, V 369, had never shown any evidence of tuberculosis and is still living 26 months after the exposure. A vaccinated animal, U 784, was killed 4.7 months after exposure and no gross tuberculosis was found anywhere in the body. The remaining 4 vaccinated rabbits showed strictly localized ulcerative pulmonary tuberculosis of varying degree of extension. In no instance were the draining tracheobronchial lymph nodes involved, and hematogenous dissemination to the rest of the body was limited to a few scarcely visible miliary tubercles in the cortex of one or both kidneys, with the single exception stated below.

The least extensive disease was seen in rabbit V 366 (Fig. 4). In its right lung there were three small, well walled off cavities, about 2-5 mm in diameter, containing tubercle bacilli. These apparently represent the original primary foci which had undergone caseation and liquefaction and had become encapsulated. There was no bronchogenic, lymphogenous, or hematogenous dissemination beyond these primary lesions when the rabbit died from renal infarction 9.5 months after infection as a result of a surgical operation.

In V 30 (Fig. 5) the disease spread by

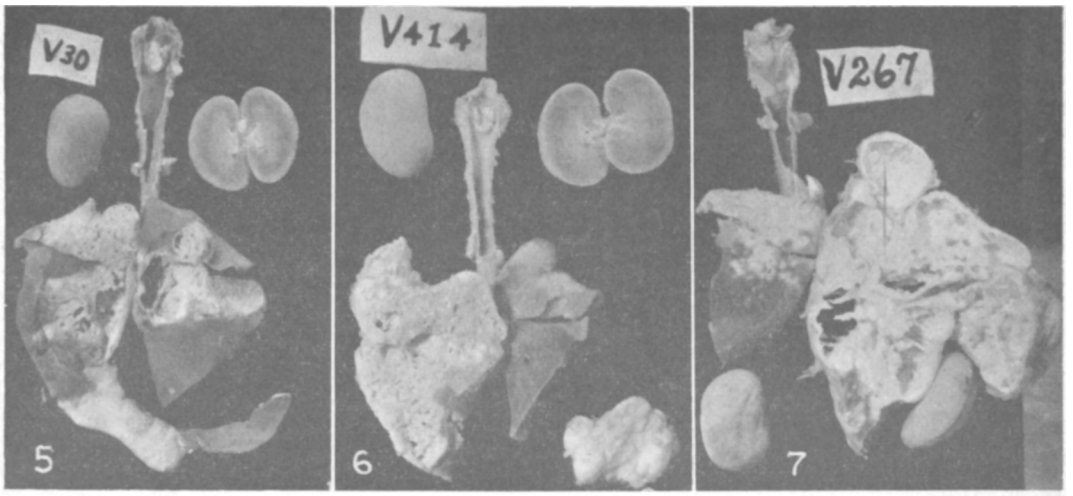

FIG. 5. The organs of rabbit V 30 at death, 10.1 months after exposure. One large cavity in each lung with limited bronchogenic spread in both. Tracheobronchial nodes and kidneys, normal. Ulcerative tuberculous laryngitis with tubular spread to the appendix, seen below left lung.

FIG. 6. Organs of rabbit V 414 at death, 7.3 months after exposure. Unilateral ulcerative tuberculosis in left lung with slight bronchogenic dissemination in the upper lobe of right lung. Tracheobronchial nodes and kidneys, normal. Ulcerative tuberculous laryngitis. Tuberculous pleural nodule in the right lower corner of photograph. The progression of the disease in this rabbit during the first 4 months of the infection is depicted in Figs. 2 and 3 above.

FIG. 7. The organs of rabbit V 267 at death, 6.6 months after exposure. Completely excavated tuberculosis of all lobes of right lung, including azygous lobe which is depicted just above the right kidney. Consolidation of upper lobe of left lung and bronchogenic spread to lower lobe of the same lung. Tracheobronchial nodes, normal. The few, minute, miliary tubercles in the kidneys cannot be seen.

bronchogenic dissemination to a considerable portion of both lungs from a single large cavity in each. The rabbit was asphyxiated by an ulcerative tuberculous laryngitis 10.1 months after infection. In the next rabbit of this series, V 414, the disease extended to a greater degree. Fig. 2 and 3 show the progression of the disease in the lungs during the first 4 months of the infection. Fig. 6 illustrates the character of the tuberculosis at death. There was a unilateral destructive phthisis with slight contralateral lesions. This rabbit also died with tuberculous, ulcerative laryngitis, 7.3 months after its infection. There was some pleural tuberculosis and one wrist joint was involved. Finally, the disease extended most rapidly in rabbit V 267, which died 6.6 months after exposure to the inhalation of 50 tubercle bacilli from complete excavation of all the lobes of the right lung including the azygous lobe. Fig. 7 illustrates the resulting thick walled cavities, the caseous consolidation of the upper lobe of the opposite lung and the bronchogenic spread to the lower lobe of the same lung.

Thus, a group of 6 rabbits of the same highly inbred, genetically resistant family showed all the degrees of resistance as seen in man; from failure of the disease to take root at all, to the formation of primary lesions which did not extend beyond their site of inception, to limited bronchogenic dissemination from the primary ulcerative foci, to unilateral ulcerative phthisis, and finally, to a rapidly progressive ulcerative tuberculosis which had destroyed one lung and was progressing from the upper to the lower lobes in the contralateral lung. The similarity of these observations to the varying types of ulcerative pulmonary phthisis as seen in man is striking.

While these animals all showed a localization of the disease which is characteristic of resistant rabbits, as evidenced by their capacity to limit the infection to the portal of entry, the lung,[6] they varied markedly in

their ability to restrict the dissemination of the disease by bronchogenic spread.

It is noteworthy that there was evidence in some of the rabbits that 50 inhaled bacilli had given rise to about 3 primary pulmonary lesions. These 3 pulmonary lesions were seen in rabbit V 366 at death and, in rabbit V 414 (Fig. 2), 3 foci were identified in the radiograph of its lungs 2 months after infection. It is noted above that an average of 9 ± 7 tubercle bacilli were necessary to generate a single tubercle in the preliminary experiments. Therefore, taking the larger figure, 16, as applicable in this series, 3 primary pulmonary foci would be expected from these 50 inhaled bacilli. The larger ratio is likely for this group since these rabbits, unlike the former animals, were slightly immunized with heat-killed tubercle bacilli prior to exposure.

Summary. A modification of an apparatus for quantitative airborne infection as devised by Wells is described. Its use has indicated that, while the number of tubercles generated in the lung is to a certain degree proportional to the number of droplet nuclei of tubercle bacilli inhaled, there is no constant ratio between the number of tubercle bacilli arrested in the lung and the number of primary tubercles generated therein, even when the rabbits breathe the same infected air simultaneously.

By exposing vaccinated highly inbred, genetically resistant rabbits to the inhalation of known small numbers of virulent bovine type tubercle bacilli it was possible to reproduce various types and phases of human localized ulcerative pulmonary tuberculosis of the adult or reinfection type. These phases ranged from complete resistance to the infection to varying degrees of bronchogenic dissemination from excavated foci to one or both lungs. There was little or no lymphogenous or hematogenous dissemination from the pulmonary portal of entry in these rabbits.

It is a pleasure to acknowledge the aid given us by Mr. R. J. Ott of the Philadelphia Gas Co., in calibrating the inclined draft gauge, and that of Mr. Peter Zappasodi for the photographs in this paper.

[6] Lurie, M. B., *Am. Rev. Tuberc.*, 1941, **44**, Suppl. 1.

16781

Failure of Antihistaminic Drugs to Reduce Reactive Hyperemia in Man.*

MILTON LANDOWNE† AND WALTER S. THOMPSON, JR.

From the Department of Medicine, University of Chicago.

The physiological response of regional dilatation consequent to circulatory arrest has been designated as reactive hyperemia. The studies of Lewis and Grant[1] and Goldblatt[2] presented evidence indicative that this is a local response and the former suggested that the accumulation of a chemical was responsible for the vasodilatation. Later Lewis[3] labeled this agent H-substance and defined it as "any substance (or substances) that is liberated by the tissue cells and exerts on the minute vessels and nerve endings an influence culminating in the triple response". Subsequently others[4–6] reported finding increased amounts

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† Present address: Cardiovascular Research Unit, Veterans Administration Hospital, Washington. D.C.

[1] Lewis, T., and Grant, R., *Heart*, 1925-26, **12**, 73.

[2] Goldblatt, H., *Heart*, 1925-26, **12**, 281.

[3] Lewis, T., The Blood Vessels of the Human Skin and Their Responses, 1927, London, Shaw and Sons.

[4] Barsoum, G. S., and Gaddum, J. H., *J. Physiol.*, 1935, **85**, 13P.