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.*

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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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[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.

of histamine, or of a substance with the "biological properties of histamine", in the venous blood during reactive hyperemia and concluded that this was responsible, at least in part, for the vasodilatation. Emmelin *et al.*[7] and Kwiatkowski,[8] using the same or modifications of the same method of analysis for histamine, were unable to confirm these results. They stated, however, that their findings did not exclude the possibility that histamine is formed *in situ* and plays a role in the development of reactive hyperemia, as the possibility remains that it might not readily diffuse away from the site of action.

Despite certain objections,[9] Lewis' studies and the subsequent work have led to the generally accepted hypothesis that the vascular response in reactive hyperemia is due primarily to the accumulation of histamine or a histamine-like substance. The recent development of the antihistaminic drugs and the establishment of their histamine antagonizing property suggested a method whereby the validity of this concept might be tested in man. While this work was in progress a report of a similarly directed study in the cat appeared.[10]

Method. Six subjects were studied. These included 5 male patients hospitalized for reasons other than cardiovascular disease and one hypertensive female patient before and 24 days after combined nephrectomy and unilateral sympathectomy. Measurement of blood flow in the foot and distal portion of the leg was made by means of a venous occlusion volume recording plethysmograph according to principles previously described.[11] This entailed placing the part of the limb to be tested within an airtight chamber. At the proximal margin of the plethysmograph a cuff 4 cm wide was placed around the leg and used to produce venous occlusion. A cuff 17 cm wide was placed about the thigh for the purpose of producing arterial occlusion. Both cuffs were attached to tanks in such a manner that by means of a system of valves any desired pressure could be rapidly obtained or released. The majority of studies were made after the subject had an average hospital breakfast, and they extended over 2 to 3 hours. Temperatures within the plethysmographic chamber were between 25.6° and 29.4° C. After at least 30 minutes of rest in the supine position determinations of resting flow were made as frequently as every 20 seconds. Reactive hyperemia was produced by the inflation and release of the arterial occlusion cuff. The cuff was rapidly inflated to approximately 220 mm Hg and the occlusion was maintained for periods of one-half to 10 minutes. Just prior to release the venous occlusion cuff was inflated and thus the initial inflow rate immediately following the release of arterial occlusion could be recorded with minimal distortion. The initial inflow rate is taken as representative of the degree of vasodilatation resulting from the occlusion. The subsidence of the vasodilatation was noted by repeating determinations of flow as often as every 15 seconds. At least one minute after subsidence of hyperemia the arterial occlusion was repeated, until a number of measurements of hyperemia were made. After completion of these control observations under normal conditions, "benadryl" (beta-dimethyl-aminoethyl-benzhydryl ether hydrochloride) 10 to 50 mg intravenously, or "pyribenzamine" (beta-dimethyl-aminoethyl - 2 - pyridyl-benzyl ammonium chloride) 50 mg orally, was administered. Observations were repeated at intervals for more than an hour.

The volume of the portion of the limb tested was measured by water displacement, and flows were expressed in terms of cc/min./100 cc limb. In 2 of the subjects intradermal injections of 0.1 cc containing 0.01 to 0.1 γ of histamine base were used to demonstrate

[5] Barsoum, G. S., and Smirk, F. H., *Clinical Science*, 1936, **2**, 353.

[6] Anrep, G. V., Barsoum, G. S., Salama, S., and Souidan, Z., *J. Physiol.*, 1944, **103**, 297.

[7] Emmelin, N., Kahlson, G., and Wicksell, F., *Acta Physiol. Scand.*, 1941, **2**, 110.

[8] Kwiatkowski, H., *J. Physiol.*, 1941, **100**, 147.

[9] Hamilton, W. F., Chapter 38 in Howell's Textbook of Physiology, edited by J. F. Fulton. 15th Ed., 1946, Philadelphia, W. B. Saunders.

[10] Emmelin, K., and Emmelin, N., *Acta Physiol. Scand.*, 1947, **14**, 16.

[11] Landowne, M., and Katz, L. N., *Am. Heart J.*, 1942, **23**, 644.

TABLE I.
Effect of Antihistaminic Drug Upon Blood Flow in the Foot-Leg at Rest and During Reactive Hyperemia.

Subject and drug*	Blood flow cc/min./100 cc limb (Each value is the average of the number of measurements indicated in parentheses)							
	Resting flow	Initial inflow after release of occlusions maintained for						
		30 sec.	1 min.	2 min.	3 min.	4 min.	5 min.	10 min.
Pr. before	3.60 (20)	5.7 (3)	6.8 (3)	7.8 (1)	8.7 (2)			
after 50 mg B	2.41 (22)	5.5 (2)	7.7 (1)	8.5 (1)	9.4 (1)			
Hu. before (right)	0.78 (21)					12.0† (1)	13.1 (2)	
after 50 mg P	1.33 (22)						12.9 (2)	
Hu. before (left)‡	6.56 (14)	15.0 (1)	12.4 (2)		12.8 (1)			
after 20 mg B	6.04 (7)	12.8 (2)	14.5 (3)		13.8 (1)			
Be. before	4.43 (8)						8.6 (1)†	10.2 (1)
after 20 mg B	3.53 (9)							10.5 (3)
Bo. before	0.76 (28)		3.1 (2)	5.3 (2)	6.3 (2)			
after 20 mg B	0.75 (19)		5.3 (2)	5.7 (1)	7.9 (1)			
Jo. before	1.35 (30)	2.0 (2)	4.0 (2)	5.4 (2)	5.5 (2)			
after 30 mg B	1.21 (9)	2.3 (4)	5.3 (2)	6.1 (2)	6.0 (2)			
Cl. before	1.45 (13)	2.4 (2)	3.2 (1)	6.3† (2)	8.4 (2)			
after 30 mg B	3.10 (11)	3.3 (2)	4.9 (3)		8.0 (1)			
Average before	2.70	6.29	5.91	6.14	8.31		13.1	10.2
after	2.62	5.98	7.54	6.77	9.00		12.9	10.5

* B = intravenous benadryl; P = oral pyribenzamine.
† Not included in average.
‡ Sympathectomized.

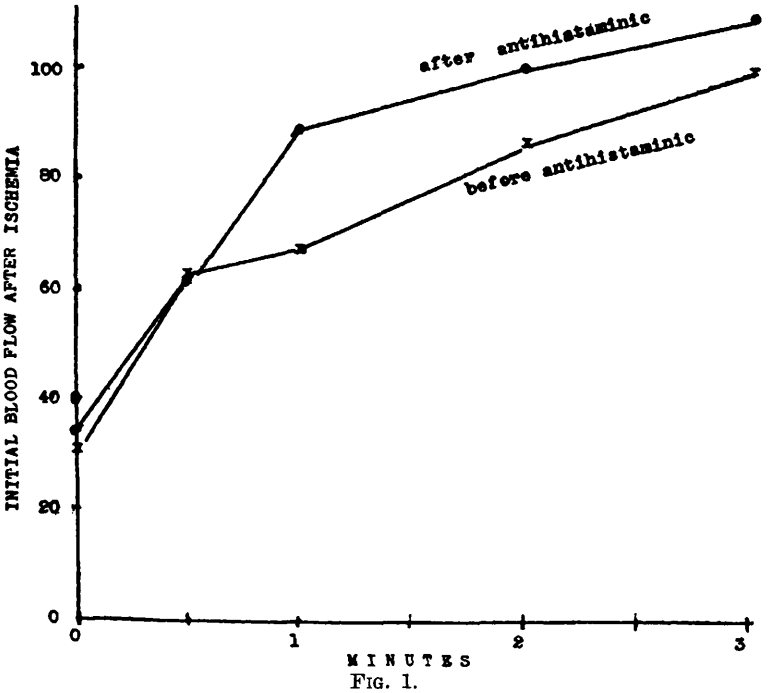

FIG. 1.

The effect of an antihistaminic drug upon blood flow at rest and during reactive hyperemia following arterial occlusion of ½, 1, 2, and 3 minutes, respectively. Averaged data from five subjects. The "control" hyperemia after three minutes of ischemia has been arbitrarily given the value of 100.

the effectiveness of the antihistamine drug. This was done simultaneously with the blood flow studies.

Results. Average blood flows recorded before and after the administration of the antihistaminic drug during the resting state and at the onset of reactive hyperemia are summarized in Table I. Each value for resting flow represents the average of seven to 30 determinations, a total of 134 before and 99 after the drug. The average difference in average resting flows before and after administration of the drug is 0.08 cc, a decrease of 3%. The average of the per cent difference in each subject is plus 16%, due to low resting control flows in two cases.

Each value for hyperemia represents the flow after either one to 4 periods of occlusion of the duration indicated. Hyperemia was measured 39 times before and 36 times after the drug. Nineteen grouped comparisons were made. In only 4 of these was a decrease in flow noted after administration of the antihistaminic drug. In all other comparisons greater hyperemic flow was recorded after giving the drug than before. The average change in hyperemia for all groups was 0.65 cc/min. (range ± 2.20 cc/min.). The average change for each subject was 0.54 cc/min. (range −0.20 to + 1.43 cc/min.); and the average change after occlusions of like duration was 0.46 cc/ min., or 5.5%. The average percentage change in each of these categories of comparison was between 10 and 15%.

In Fig. 1 are presented the averaged results in 5 experiments before and after the administration of the antihistaminic drug comparing flow at rest and during reactive hyperemia where arterial occlusions of one-half, one, 2 and 3 minutes respectively were used. The initial hyperemic flow after 3 minutes of occlusion before the drug was given is arbitrarily taken as a comparison standard of 100.

The duration of reactive hyperemia was brief, particularly after the shorter occlusions. Because of the normal fluctuations in resting

TABLE II.
Effect of Antihistaminic Drug Upon Whealing Produced by Intradermal Histamine.
(0.1 cc of a concentration of 1.0 γ per cc of histamine base was injected intradermally before and 17 min. after 30 mg of "Benadryl" intravenously.)

Min. after injection		Before drug	After drug
3	size of wheal, mm	25 × 14	15 × 12
	" " erythema, mm	30 × 35	20 × 20
13	wheal	22 × 20	15 × 14
	erythema	40 × 45	33 × 33
23	wheal	22 × 20	15 × 12
	erythema	47 × 55 intense	33 × 33 fading

flow, quantitation of either the total amount or of the duration of the increased circulation is not feasible under these conditions. No qualitative differences were observed in these aspects of reactive hyperemia after antihistaminic drug.

In comparing the period before administration of the antihistaminic drug with that afterward it will be noted that no significant effect of the drug upon blood flow is demonstrated. In some experiments there was a slight average increase in resting flow, and a comparable average increase in hyperemic flow. The air temperature within the plethysmographic chamber rose 0.5° to 2.5°C, an average increase of 1.1°C. The increase in local temperature, further relaxation of the subject, and any effects of specific dynamic action are factors which may account for a slight increase in flow during the latter part of an experiment.

The effectiveness of the antihistaminic agents used is indicated by the illustrative experience presented in Table II. The wheals and surrounding erythematous halo were smaller in size and persisted for a shorter period of time when histamine in graded dosage was injected after the administration of the drug.

Discussion. The data presented demonstrates the failure of antihistaminic drugs to reduce the vasodilatation of reactive hyperemia in the human limb. This confirms the work of Emmelin and Emmelin[10] who were unable to show in the cat that antihistaminic drugs reduced the hyperemic volume increase following circulatory arrest.

In our studies an antihistaminic drug was carried to the tissues by means of the circulation. The concentration attained was sufficient to decrease the *local* intradermal whealing effect of 0.1 cc of concentration of 1.0 γ per cc of histamine base. Lewis considered[3] that histamine introduced into the skin acted upon the walls of the minute vessels. Emmelin and Emmelin[10] showed that the effect of intra-arterial histamine upon the minute vessels of the cat's hind limb could be reduced by an antihistaminic drug. Thus *exogenous* histamine, applied either from the outside or from within the lumen of the vessel, may have its effect upon the vessel wall reduced by the antihistaminic drug. The mechanism of the histamine blocking ability of these drugs is not known, but it has been suggested[12] that it is due to a competitive or combining property of the drug at the site of action of histamine rather than with histamine itself. This site of action would be at some portion of the cell surface or within the particular cells concerned in effecting the response. The inhibition of the action of *exogenous* histamine by an antihistaminic drug would indicate that the antihistaminic had reached this site of action. In this event, the effect of *endogenous* histamine, if produced outside or within the particular cells of the blood vessels which cause the vasodilatation of reactive hyperemia, should also be inhibited by circulating antihistaminic drugs.

The alternative possibility, that the effector

[12] Friedlaender, S., and Feinberg, S. M., *J. Allergy*, 1946, **17**, 129.

cell is not penetrated by the antihistaminic drug, requires that the site of action of histamine be at a surface to which *exogenous* histamine is carried, by the circulation, by penetration through cells, or by passage between cells. In this circumstance it is conceivable that *endogenous* histamine may, by arising within the cell, reach and act upon the surface in a manner not open to inhibition by the agent used. This is unlikely. "Benadryl" is a small, ionizable molecule which readily passes from the circulating blood to the extracellular spaces. There is no evidence to indicate that it is unable to traverse the cellular structure of the vessel wall in its passage. Clinical studies[13] have shown that those allergic manifestations held to be due to an increase in *endogenous* histamine activity are reduced by antihistaminic drugs. This requires that the drug reach the site of action of the *endogenous* histamine liberated in these disorders.

The term "antihistaminic" indicates that these drugs possess the ability to inhibit the biological effects of histamine. It would be expected that a "histamine-like" substance "with the biological properties of histamine" would be similarly inhibited. This cannot be determined, of course, until such substance is actually demonstrated.

Thus the failure of antihistaminic drugs to reduce the vasodilatation of reactive hyperemia in man can be considered to mitigate strongly against the generally accepted concept that histamine or a histamine-like substance is the primary factor in the vasodilatation of reactive hyperemia.

The unmodified persistence of the capacity for reactive hyperemia after sympathetic denervation indicates, as Lewis pointed out,[3] that this phenomenon is not dependent upon sympathetic integrity. The antihistaminic drug failed to modify reactive hyperemia in the sympathectomized limb. This demonstrates that the drug does not normally tend to evoke a compensatory reduction in sympathetic activity which could mask an effect on reactive hyperemia.

Summary and Conclusions. 1. In 6 subjects reactive hyperemia was produced in the legs by release of arterial occlusion maintained for from one-half to 10 minutes. Blood flow in the foot and distal portion of the leg was measured with a venous occlusion plethysmograph. The initial inflow rate immediately following release of arterial occlusion is taken as representative of the degree of vasodilatation resulting from the occlusion.

2. After administration of effective amounts of antihistaminic drugs the reactive hyperemia was not diminished.

3. The mechanism of reactive hyperemia has not been shown to be due to release of histamine or "H-substance." It is as yet unexplained.

[13] Feinberg, S. M., *J. A. M. A.*, 1946, **132**, 702.

16782 P

Nucleic Acid Content of Chromosomes of Normal and Leukemic Mouse Spleen.*

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It has recently been shown by Mirsky and Ris[1] that chromosomes may be isolated from the resting nucleus. These isolated chromosomes are made up chiefly of desoxypentose nucleic acid (DNA), histone, and the

* The expenses of this investigation were defrayed by grants from the Office of Naval Research, the National Cancer Institute of the United States Public Health Service, the Finney-Howell Foundation, and the James Foundation of New York, Inc.

[1] Mirsky, A. E., and Ris, H., *J. Gen. Physiol.*, 1947, **31**, 1, 7.