TABLE II. Fatty Acid Composition of Cholesterol Esters from Aortas with Different Degrees of Atherosclerosis.

Grade	No. of samples	% of major fatty acids: Palmitic	Palmitoleic	Oleic	Linoleic	Eicosatrienoic?	Arachidonic
0	4	12.4	3.3	30.6	41.2	3.0	6.5
1	3	13.4	4.8	29.2	42.7	1.1	6.8
2	4	10.6	3.4	27.2	47.6	2.4	7.5
3	2	12.6	3.2	26.3	49.3	1.0	5.6
4	4	13.1	5.6	27.4	42.1	1.6	7.3

study should reveal whether these differences may be explained by changes in diet.

Summary. Analysis of lipids of the intima and media of aortas with progressive stages of atherosclerosis reveals an increase in sterol and sterol ester fractions and a decrease in phospholipids. It is proposed that these changes reveal a very low rate of lipid turnover in this tissue. The fatty acid composition of the cholesterol esters did not change with advancing atherosclerosis.

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1. Page, I. H., *Ann. Int. Med.,* 1941, v14, 1741.
2. Duff, G. L., McMillan, G. C., *Am. J. Med.,* 1951, v11, 92.
3. Gould, R. G., *ibid.,* 1951, v11, 209.
4. Hirsch, E. F., Weinhouse, S., *Physiol. Rev.,* 1943, v23, 185.
5. Shore, M. L., Zilversmit, D. B., Ackerman, R. F., *Am. J. Physiol.,* 1955, v181, 527 but see Azarnoff, D. L., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 680.
6. Page, I. H., *Ann. Int. Med.,* 1941, v14, 1741.
7. Sinclair, H. M., *Lancet,* 1956, v270, 381.
8. Tuna, N., Reckers, L., Frantz, I. D., Jr., *Proc. Am. Soc. Clin. Invest.,* 1957, v36, 932, *J. Clin. Invest.,* 1958, v37, 1153.
9. Luddy, F. E., Barford, R. A., Reimenschneider, R. W., Evans, J. D., *J. Biol. Chem.,* 1958, v232, 843.
10. Böttcher, C. J. F., Woodford, F. P., Ter Haar Romeny, C. Ch., Boelsma, E., Van Gent, C. M., *Nature,* 1959, v183, 47. See also: Böttcher, C. J. F. in program of Symposium on Drugs Affecting Lipid Metabolism, Milano, Italy, June 2-4, 1960, p. 29.
11. Swell, L., Field, H., Schools, P. E., Jr., Treadwell, C. R., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 651.
12. Fillerup, D. L., Mead, J. F., *ibid.,* 1953, v83, 574.
13. Smith, E. G., *Biochem. J.,* 1959, v73, 34P.
14. Glavind, J., Hartmann, S., Clemmeson, J., Jesson, K., Dam, H., *Acta Pathol. Microbiol. Scand.,* 1952, v30, 1.

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A Ventilatory Effect of Carbonic Anhydrase Inhibition in Man. (26219)

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An increase in resting ventilation has been a consistent finding in dogs given the carbonic anhydrase inhibitor acetazolamide(1, 2,3). In man, however, no increase in resting ventilation has been reported even though doses of the drug were well within the range which produced a marked effect in dogs(4,5, 6). In seeking a reason for the absence of an effect in man in the reports, it was noticed that the drug was usually given intravenously into the dogs but invariably was taken orally by the human subjects. If insufficient time during the experiment was allowed for absorption and attainment of a sufficient concentration of drug in the blood, the ventilatory response of man to carbonic anhydrase inhibition might well not have been seen. In the experiments described below, the results obtained in man with the same dose of drug given orally and intravenously are compared.

Methods. The subjects were 5 normal males ranging in age from 21 to 36 years. To collect expired gas, the subject wore either a rubber gas mask with a hose adapter on the expired valve port or a mouthpiece and valve arrangement and a nose clip. The expired gas was led through a mixing cylinder of 1.5 liters and from there through a dry test gas meter. The mixed expired gas sample was taken through a 13 gauge needle inserted into the rubber tubing at a point adjacent to the distal opening of the mixing chamber. The sample gas was pulled serially at a known rate through a drying tube, a Beckman Model E3 oxygen analyzer, and a Liston-Becker Model 16 CO_2 analyzer. From readings of the dry test gas meter and the gas analyzers, the minute volume of ventilation, CO_2 output and oxygen uptake were calculated. Frequency of respiration was also recorded.

Experiments were always run in the morning. The subject rested in the supine position on a cot for approximately 30 minutes prior to beginning the experiment. The gas mask or valve and nose clip were adjusted during this period and the subject was allowed at least 10 minutes to become accustomed to it. Control measurements were made for 30 minutes after beginning the experiment. The dry test meter was read every 6 minutes during which gas analyzers were read every 2 minutes and the 3 readings were averaged for the 6-minute period. Frequency of respiration was recorded during second and fifth minutes and averaged.

At the end of control period, the subject either ingested acetazolamide in tablet form or the sodium salt of the drug was infused in saline into an antecubital vein. In both cases, subject received 25 mg/kg of body weight. For the infusion, the requisite amount of drug was dissolved in approximately 250 cc of physiological saline and was allowed to drip into the vein over a period of 12 minutes. Every subject received the drug by both routes in 2 separate experiments. In the experiments in which drug was administered orally, data were collected for 3 hours after ingestion. When the drug was given intravenously, experimental period was continued for 2 hours after beginning of infusion.

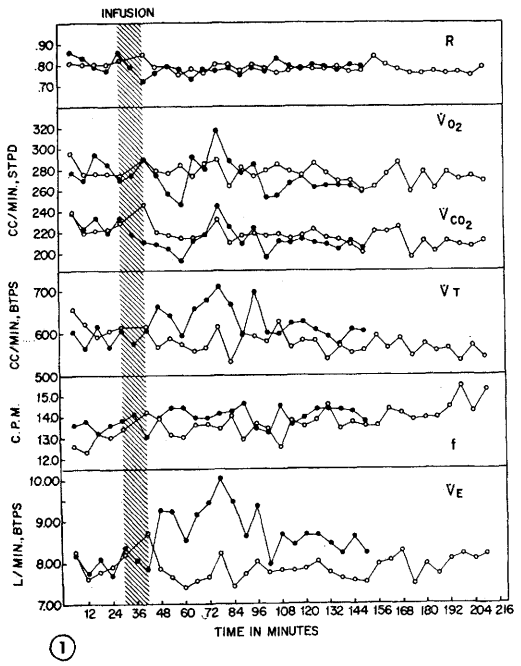
Two of the subjects, in a third experiment, were given 50 mg/kg of acetazolamide intravenously. The same procedure as described for the lower dose was followed.

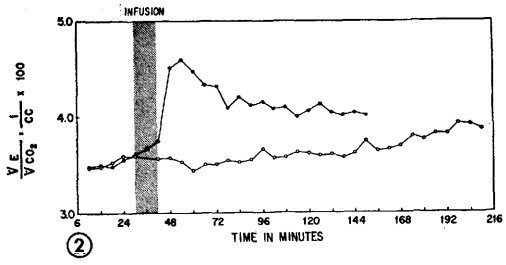
Results. Mean values of O_2 consumption, CO_2 output, and respiratory exchange ratio of the 5 subjects are plotted against time in experiment in Fig. 1. There appeared to be no consistent difference in metabolic gas exchange as a result of different routes of administration of acetazolamide.

Ventilation at the bottom of Fig. 1, increased approximately 15% at the end of intravenous infusion of acetazolamide. Approximately one hour later, ventilation had returned almost to control level although it was still slightly elevated. No such increase was seen with the oral dose. Mean values of tidal volumes, also in Fig. 1 indicated that the increased ventilation after intravenous injection of acetazolamide was primarily due to an increased tidal volume.

As a more sensitive reflection of ventilatory status, ratio of ventilation to CO_2 output (the ventilatory equivalent for CO_2) was calculated and mean values for the 5 subjects are shown in Fig. 2. Approximately 2 hours after ingestion of drug, this ratio began to increase and showed evidence of a continuing increase 3 hours after ingestion. Means of the 5 determinations for each subject during control period and the last 30 minutes of experimental period were compared in a paired t-test by taking the difference for each subject. The increase in the ventilatory equivalent for CO_2 during the last 30 minutes was highly significant ($P<.01$). The venous route of drug administration produced even more pronounced effects on the ventilatory equivalent for CO_2. Comparing the period of maximum effect, the first 30 minutes after end of infusion, with the control period, the ratio was increased over 25%. After reaching a maximum, the ratio declined and approached that obtained with the oral drug, in both cases greater than during the control.

In 2 subjects given 50 mg/kg of acetazolamide intravenously, the results did not differ notably from those of the lower dose given intravenously. Mean values of the ventilatory





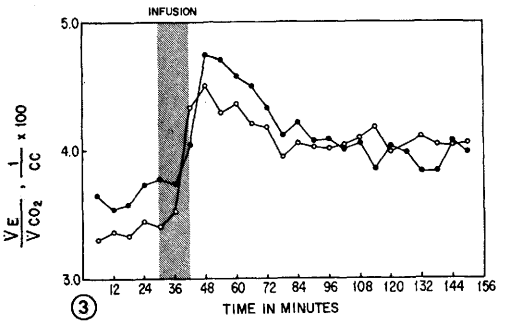


FIG. 1. Mean values of respiratory exchange ratio (R), oxygen uptake ($\dot{V}_{O_2}$), CO_2 output ($\dot{V}_{CO_2}$), tidal volume ($\dot{V}_{T_{BTPS}}$), respiratory frequency (f), and expired minute volume of ventilation ($\dot{V}_{E_{BTPS}}$) of 5 male subjects who were given 25 mg/kg of acetazolamide orally (○) and intrav. (●).

FIG. 2. Mean values of ventilatory equivalent for CO_2 ($\dot{V}_E/\dot{V}_{CO_2}$) of 5 subjects. ○, oral dose of 25 mg/kg; ●, intrav. dose of 25 mg/kg.

FIG. 3. Mean values of ventilatory equivalent for CO_2 of 2 subjects who were given 25 mg/kg of acetazolamide intrav. (●) and 50 mg/kg intrav. (○).

equivalent for CO_2 for the 2 subjects with the 2 doses are shown in Fig. 3 and serve to illustrate this point. Similar correspondence was seen in comparisons of the other variables.

Discussion. In dogs, inhibition of carbonic anhydrase caused a transient reduction in CO_2 output and an increase in ventilation (1), or only an increase in ventilation with no change in CO_2 output(3). For the purpose of this discussion, therefore, any significant increase in ratio of ventilation to CO_2 output (the ventilatory equivalent for CO_2), was considered to be a ventilatory effect of carbonic anhydrase inhibition.

Acetazolamide given intravenously produced a marked increase in ventilation and the ventilatory equivalent for CO_2 The same dose taken by mouth, however, did not have an equal effect on ventilation and the effect on the ventilatory equivalent was much delayed and smaller in magnitude (Fig. 2). The delay of approximately 2 hours was probably related to time required for absorption of the drug into the bloodstream and build-up of a suitable concentration there. Two hours corresponds fairly well with the data of Maren and Robinson(7) who obtained peak plasma concentrations of drug at that time in patients given 75-85 mg/kg by stomach tube.

The increase in ventilation caused by intravenous administration of acetazolamide was more in keeping with results obtained from dogs. The magnitude of the increase, however, was much less. Both anesthetized and non-anesthetized dogs increased ventilation by approximately 100%(1,2,3). Maximum increase was only 15% in these experiments on man. The fact that doubling the dose in 2 subjects did not produce a greater effect tended to rule out the possibility that carbonic anhydrase had not been completely inhibited. If inhibition was complete, then the results in man suggest a lesser role of the enzyme in man compared to the dog.

It has been shown that in the dog, bicarbonate transport of CO_2 was reduced from 58% to 17% of total CO_2 exchanged at the lung after inhibition of carbonic anhydrase(8). This necessitated that a greater proportion

of CO_2 must be transported in carbamino combinations and in physical solution to maintain a steady state. For this to take place, the gradient for CO_2 from the tissues to alveolar air must have been increased, particularly in the case of CO_2 in physical solution. To accommodate for the inhibition of carbonic anhydrase, the subjects in these experiments neither increased their ventilation as much nor retained as much CO_2 as did anesthetized dogs(1). This implied that alveolar PCO_2 was not lowered to the same extent nor was tissue PCO_2 raised to the same extent. Since carbamino transport of CO_2 is less subject to changes in PCO_2 than the transport of CO_2 in physical solution(9), a possible explanation for the different responses of man and the dog to carbonic anhydrase inhibition was that a greater proportion of CO_2 exchanged at the lung was derived from CO_2 in carbamino combinations. It must be stressed, however, that this is purely speculative.

Aside from raising an interesting question about CO_2 transport, this study has demonstrated a ventilatory effect of carbonic anhydrase inhibition in man when the inhibitor was given orally as well as intravenously. The delay in effect of an oral dose, due probably to absorption time, has been pointed out as a possible pitfall for studies in man. Whether or not a species difference exists for the relative importance of carbonic anhydrase in CO_2 transport remains to be settled.

Summary. Five normal males were given 25 mg/kg of the carbonic anhydrase inhibitor acetazolamide both orally and intravenously in separate experiments. The oral dose caused a significant increase in ratio of ventilation to CO_2 output, but only after a delay of approximately 2 hours after ingestion. The intravenous dose caused a greater increase in ratio of ventilation to CO_2 output which was evident as soon as infusion of drug was completed. It was concluded that carbonic anhydrase inhibition in man did produce a ventilatory effect which had not been demonstrated previously when the inhibitor was given orally.

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1. Cain, S. M., Otis, A. B., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 439.
2. Carter, E. T., Clark, R. T., Jr., *J. Appl. Physiol.*, 1958, v13, 42.
3. Mithoefer, J. C., *ibid.*, 1959, v14, 109.
4. Becker, E. L., Hodler, J. E., Fishman, A. P., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 193.
5. Cranston, W. I., Sanderson, P .M., Stapleton, T., *J. Physiol.*, 1955, v129, 71p.
6. Stapleton, T., Cranston, W. I., Cross, K. W., Trythall, D. A. H., *Helv. Acta Paed.*, 1955, v10, 210.
7. Maren, T. H., Robinson, B., *Bull. Johns Hopkins Hosp.*, 1960, v106, 1.
8. Cain, S. M., Stainsby, W. N., Otis, A. B., *Fed. Proc.*, 1959, v18, 22.
9. Ferguson, J. K. W., *J. Physiol.*, 1936, v88, 40.

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Effect of Pitressin on Cationic Exchange and Muscular Activity in the Dibenzylene Blocked Rat Gastrocnemius.* (26220)

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We have previously reported that in rats, high doses of adrenergic blocking agents diminish running ability as measured on a treadmill(1). With this loss of ability to perform work the transmembrane cationic exchanges ordinarily mirrored in the plasma in association with muscular exercise are also proportionately reduced. Since these adrenergic blocking agents are equally effective in stopping contraction of the isolated rat diaphragm

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