the seminal vesicles produced 4 lines obtained with seminal plasma, *i.e.*, all of the reactions of seminal plasma except the one indicated by innermost (nearest to antiserum trough) weak line. Serum, kidney and liver extracts did not produce lines of precipitation.

Conclusions and summary. 1) Comparison of data with those on human semen presented previously(4) shows a close similarity in the immunological properties of seminal products of man and rabbit. In both species, seminal plasma and spermatozoa contain powerful antigens, so closely related that they have resisted differentiation. 2) These antigens are highly species and organ specific. Such cross-reactions as could be obtained, according to evidence provided by agar diffusion method, are due to one of the 5 components demonstrable by this procedure. The multiplicity of antigenic components corresponds with that previously shown in man and guinea pig (4-6). 3) Data obtained from human semen specimens lacking spermatozoa suggested that the antigenic material does not originate with spermatozoa. This view is further strengthened by the low degree of cross reactivity of rabbit sera with extracts from testes and epididymis, and, still more strongly, by evidence that extracts of seminal vesicle wall contain some antigenic components reactive with anti-seminal plasma and anti-spermatozoal immune sera. The seminal vesicle fluid of the rabbit contains in abundance nearly all antigenic components demonstrable with our sera. 4) The effective antigens found in seminal plasma and spermatozoa of semen appear to originate in the seminal vesicle.

1. Henle, W., *J. Immunol.,* 1938, v34, 325.
2. Henle, W., Henle, G., Chambers, L. A., *J. Exp. Med.,* 1938, v68, 335.
3. Smith, A. U., *Proc. Roy. Soc. London,* 1950, Ser. B, v136, 46.
4. Weil, A. J., Kotsevalov, O., Wilson, L., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 606.
5. Pernot, E., *Bull. Soc. Chim. Biol.,* 1956, v38, 1041.
6. Ross, V., *J. Immunol.,* 1946, v52, 87.
7. Larson, B. L., Gray, R. S., Salisbury, G. W., *J. Biol. Chem.,* 1954, v211, 43.
8. Macirone, C., Walton, A., *J. Agric. Sc.,* 1938, v28, 122.
9. Gregoire, A. T., Bratton, R. W., Foote, R. H., *J. Animal Sc.,* 1958, v17, 243.
10. Woodehouse, R. P., *Ann. Allergy,* 1956, v14, 96.
11. Katsh, S., *J. Exp. Med.,* 1958, v107, 95.
12. Landy, M., Trapani, R. J., *Am. J. Hyg.,* 1954, v59, 150.
13. Wilson, L., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 652.

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Inhibition of Carbonic Anhydrase: Effect on Tissue Gas Tensions in the Rat* (24189)

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The established importance of carbonic anhydrase in CO_2 transport implies impairment in CO_2 elimination when the enzyme is inhibited. This controversial problem, has been discussed, and evidence presented for impaired CO_2 transport with resultant retention of this gas following carbonic anhydrase inhibition in the dog(1). The evidence presented, however, did not demonstrate a rise in venous or tissue CO_2 tension following inhibition of carbonic anhydrase, a change implicit in the mechanism of CO_2 retention from any cause. Such demonstration was not possible because usual technics for measurement of venous CO_2 tension are inapplicable in the presence of carbonic anhydrase inhibition. When carbonic anhydrase is inhibited a dis-

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equilibrium develops between CO_2 and carbonic acid and hence between CO_2 and bicarbonate. Measurements of CO_2 tension in blood performed *in vitro* allow time for equilibration to occur in the absence of enzyme activity and hence give false values for P_{CO_2} when carbonic anhydrase is inhibited. In addition, use of the Henderson-Hasselbalch equation is inapplicable under circumstances of carbonic anhydrase inhibition because its derivation is predicated on the near instantaneous equilibrium between CO_2 and carbonic acid made possible by carbonic anhydrase(2). In contrast to difficulties in measurement of P_{CO_2} in venous blood, arterial CO_2 tension can be estimated by analysis of alveolar air, since lungs afford a natural tonometer whose CO_2 tension reflects that of the gas as it existed in arterial blood leaving the lungs. The composition of an enclosed, gas-filled space within the body is known to approach equilibrium with venous blood perfusing it, hence its gas tensions approach those of adjacent tissues(3,4). Analysis of a well established, vascularized subcutaneous gas pocket in the rat was a convenient means of estimating gas pressures of venous blood perfusing the pocket(4). We report here the use of an artificially constructed tonometer on the venous side of the circulation which allows estimation of CO_2 tension of tissue and venous blood under peculiar circumstances of carbonic anhydrase inhibition when such measurements are otherwise impossible.

Methods. A. *Effect of carbonic anhydrase inhibition on gas composition of subcutaneous pockets.* Subcutaneous gas pockets were established in 10 adult Wistar rats by injection of 20 cc of air into the subcutaneous tissue of the interscapular fossa(4). After 2 weeks, when control analyses indicated a steady state, one-half the rats were injected intraperitoneally with 50 mg/kg body weight of acetazolamide† in a volume of 2 cc at a pH of 10. The other rats were given a control substance, CL 13,850‡ (2-acetylamino-1,3,4-thiadiazole-5-sulfon-*t*-butylamide), by the same route in equal molar dosage at same pH. This substance is very similar to acetazolamide in its physical properties such as molecular weight and acid dissociation constants but has only 4×10^{-5} times the inhibitory effect on carbonic anhydrase. Gas samples were obtained on all rats at intervals to 90 minutes after injection and analyzed using a Scholander micro gas analyzer. Measurement of blood carbonic anhydrase activity before and 30 minutes after injection of the 2 substances was carried out in comparable group of 6 rats (5). B. *Effect of carbonic anhydrase inhibition on pulmonary ventilation.* Six Wistar rats weighing 250-300 g were anesthetized with pentobarbital injected intraperitoneally in dose of 29 mg/kg. The tracheae were cannulated and ventilation measured by a recording microspirometer containing oxygen. CO_2 was absorbed by a sodium hydroxide wick within the spirometer. After ventilation had become steady acetazolamide was injected intraperitoneally in 50 mg kg in volume of 1 cc. Ventilation was measured thereafter at intervals to 30 minutes. Measurement was made for one minute, the spirometer then flushed with oxygen. The effect of anesthesia alone on ventilation was measured by this same method using intraperitoneal injection of 1 cc .85 N saline as control. *C. Effect of carbonic anhydrase on CO_2 content of arterial blood.* CO_2 content of arterial blood as well as gas tensions of subcutaneous pockets were measured in 16 other rats, following administration of acetazolamide and CL 13,850. After control samples of well established pockets indicated a steady state, the drugs were injected intraperitoneally each to ½ the animals in dose described. Thirty minutes later, (the time of maximal change), the pockets were reanalyzed, then animals were struck on head. The chest was quickly opened and 2-3 ml of blood were withdrawn anaerobically from left ventricle which had not yet stopped beating. The time from the blow to completion of sampling was standardized to 1½ minutes. The hematocrit was measured by a micro-pipette technic.

Results. A. *Gas tensions in pockets.* The CO_2 tension of gas pockets rose progressively

† Diamox was supplied through the courtesy of Lederle Lab. Division, American Cyanamid Co.

‡ Supplied through same company.

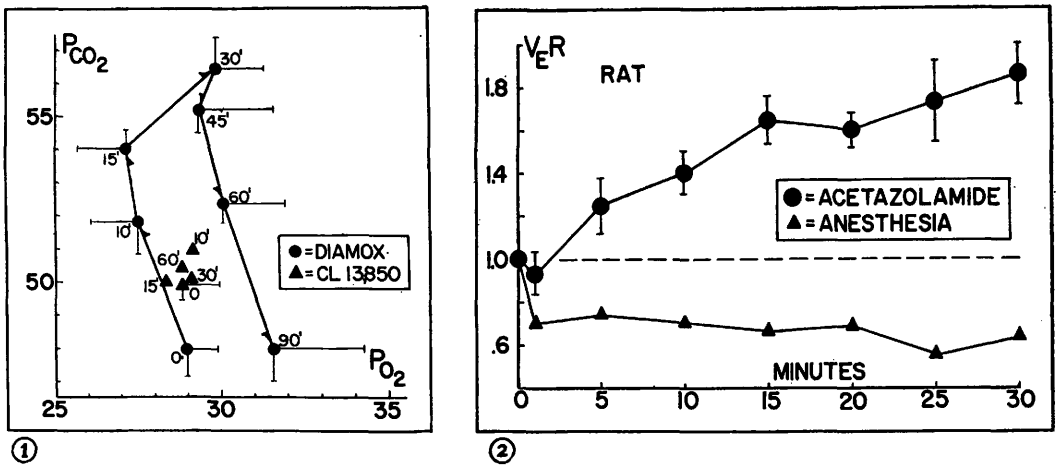

FIG. 1. Simultaneous CO_2 and O_2 tensions of subcut. pockets after acetazolamide (50 mg/kg I.P.) and CL 13,850. Times after inj. are shown. Lines radiating from points represent stand. error of mean. These lines are drawn on only 2 sides of each point to simplify the illustration.

FIG. 2. Effect of acetazolamide (50 mg/kg I.P.) and pentobarbital anesthesia (29 mg/kg I.P.) on pulmonary ventilation of the rat. Ventilation is expressed as ratio of expired minute volume to control value (V_ER). Vertical lines represent stand. error of mean.

for 30 minutes after acetazolamide injection, reaching a mean of 8.6 mm Hg above pre-injection value. It then fell, gradually returning to normal by 90 minutes. There was no significant concomitant change in oxygen tension. Injection of the control substance CL 13,850 produced no significant change in gas composition of similar pockets. These results are shown in Fig. 1. The dose of acetazolamide in these experiments resulted in 98% inhibition of carbonic anhydrase activity in blood 30 minutes after injection as tested by the method described above. Thirty minutes after CL 13,850 there was no measurable enzyme inhibition.

B. *Ventilation.* The effect of acetazolamide on pulmonary ventilation is shown in Fig. 2 where volume of expired air is compared to mean of 3 control values and expressed as a ratio (V_ER). Following a short-lived depression, there was a progressive rise in ventilation for 30 minutes. Ventilatory frequency did not change significantly, therefore the rise in minute volume represented an even greater increase in alveolar ventilation. Pentobarbital anesthesia alone produced moderate but progressive ventilatory depression as shown in Fig. 2.

C. *Arterial CO_2 content.* The CO_2 contents of arterial blood in normal rats, in those following CL 13,850, and after acetazolamide are shown in Table I. The value of 56 volumes % CO_2 in animals which were not injected is somewhat high as a result of the method of sampling. Acetazolamide injected at pH 10 resulted in statistically significant rise of 4 volumes % in arterial CO_2 content. The arterial CO_2 content was not changed following CL 13,850. This absence of effect was predictable since CL 13,850 produced no measurable change in carbonic anhydrase activity and since total amount of NaOH given with it at pH 10 was approximately 0.1 meq. The effects of acetazolamide and CL 13,850 on gas tensions of pockets were similar to the 30 minute values in the first series. There was no significant difference in values for hematocrit as measured in the 3 groups of this series.

Discussion. If it be assumed that a sub-

TABLE I. Arterial CO_2 Content and Hematocrit of Normal Rats and Those 30 Min. after Injection of Acetazolamide and CL 13,850.

	#	Arterial CO_2 vol, %	Hct.
Acetazolamide	12	60.6 (.4)	52 (1.6)
CL 13,850	11	57.7 (.8)	51 (1.3)
Normal	6	56.4 (1.6)	48 (2.5)

Numbers in parentheses are stand. errors of mean.

cutaneous gas pocket approaches equilibrium with venous blood(4), then a rise of CO_2 tension in the pocket may result from one or a combination of the following independent variables:

1) Depression of alveolar ventilation. 2) Decrease in blood flow. 3) Increase in metabolic CO_2 production. 4) Interference in CO_2 transport mechanism.

The mechanism of increase in CO_2 tension of subcutaneous pockets following inhibition of carbonic anhydrase will be discussed in terms of these 4 variables.

1. Ventilatory depression with resultant rise in arterial CO_2 tension would increase P_{CO_2} in venous blood and in gas pocket unless blood flow increased sufficiently to compensate for rise in arterial blood. This mechanism can be safely dismissed since hyperventilation followed acetazolamide, albeit, these animals were anesthetized while those in which the gas pocket composition was studied were not. This difference in experiment design was necessary since measurement of ventilation in unanesthetized rat is technically unsatisfactory. It is improbable that anesthesia belied the effect of acetazolamide in the conscious animal. The rise in arterial CO_2 content with hyperventilation is interesting. We observed this response in the dog following carbonic anhydrase inhibition when renal excretion of base is prevented by ureteral ligation. If the venous P_{CO_2} increases following carbonic anhydrase inhibition, there must be a slow approach toward equilibrium between dissolved CO_2 and carbonic acid during the time for transport of venous blood from tissues to lungs. The slight increase in bicarbonate which results would not be lost as P_{CO_2} falls during rapid passage of blood through pulmonary capillaries. Neither would the reaction be completely reversed in arterial blood before it reaches the tissues, since time of transport of arterial blood from lungs to tissues is less than circulation time of venous blood from tissues to lung. The net effect is that since volume of venous blood is greater than the arterial, at any time more blood is exposed to high CO_2 tension (venous) than to low (arterial). This must result in a gradual increase in bicarbonate in both arterial and venous blood in the absence of carbonic anhydrase activity, unless there is concomitant selective elimination by the kidney. In the rat the rise in arterial CO_2 content is consistent with CO_2 retention in the presence of disequilibrium between CO_2 and carbonic acid and suggests that renal excretion of base had not occurred by 30 minutes.

2. A rise in venous CO_2 tension secondary to a decrease in blood flow must be associated with a fall in oxygen tension. Degree of change would depend upon relative shapes of dissociation curves for CO_2 and O_2 over involved area of pressure change. The available data for rat blood(6) indicate that the observed rise in venous CO_2 tension from 48 to 57 mm Hg would be associated with an extreme fall in oxygen tension almost to zero, its precise value being a function of the exchange ratio. No significant change in oxygen tension of the pocket occurred following acetazolamide. This difference in behavior of the 2 gases was apparently not the result of a difference in rates of exchange between blood and pocket since there was no trend toward a rise in oxygen tension during the 90 minute period. It is therefore concluded that elevation of CO_2 tension was not the result of a decrease in perfusion.

3. The observed rise in CO_2 tension of the pockets cannot be explained as a result of increase in metabolic CO_2 production since there would be, of necessity, an associated increase in oxygen uptake with consequent fall in its tension in venous blood and therefore in the pocket. No such change in oxygen tension occurred.

4. A rise in venous CO_2 tension resulting from disequilibrium between CO_2 and carbonic acid in the erythrocyte is an adequate explanation of the results of these experiments. CO_2 carried by venous blood from tissues is normally transported at a tension which is dependent upon rapid equilibration between dissolved CO_2 and bicarbonate made possible by activity of carbonic anhydrase. The high tension of CO_2 which develops in the pocket and venous blood when enzyme is inhibited can be interpreted as reflecting a

failure in conversion of CO_2 to bicarbonate in the erythrocyte, more being therefore transported in the dissolved and carbamino forms at a higher pressure. The consequences of such increase in venous CO_2 tension are those which have been observed following carbonic anhydrase inhibition in the dog(1). The higher tension of CO_2 in venous blood must result in retention of CO_2, the quantity being dependent upon level of pressure and tissue dissociation constants. This equilibration at a higher level of P_{CO_2} can be accomplished by tissues because they are not dependent upon enzymatic acceleration for conversion of CO_2 to bicarbonate, since the reaction is not temporally limited by circulation. CO_2 is therefore retained until its tissue stores are increased to a new state of equilibrium.

Summary. 1. Venous and tissue gas tensions in the rat have been estimated by use of artificially constructed subcutaneous gas pocket. Inhibition of carbonic anhydrase by acetazolamide results in a rise in venous and tissue CO_2 tension independent of change of oxygen tension. This effect is associated with hyperventilation and an increase in arterial CO_2 content. 2. These findings are interpreted as direct evidence of CO_2 retention resulting from interference in CO_2 transport by carbonic anhydrase inhibition.

1. Mithoefer, J. C., *J. Appl. Physiol.*
2. Mithoefer, J. C., Mayer, P. W., Stocks, J., *Fed. Proc.*, 1957, v16, 382.
3. Tenney, S. M., Carpenter, F. G., Rahn, H., *J. Appl. Physiol.*, 1953, v6, 201.
4. Rahn, H., *Fed. Proc.*, 1957, v16, 685.
5. Philpot, F. J., Philpot, J. S. L., *Biochem. J., Lond.*, 1936, v302, 2191.
6. Sherwood Jones, E., Maegraith, B. G., Sculthorpe, H. H., *Ann. Trop. Med. Paras.*, 1950, v44, 168.

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Neutrophil Life Cycle with Tritiated Thymidine.* (24190)

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Patterns of neutrophil balance can be brought into sharper focus than heretofore possible by means of high resolution radioautography with tritium-labeled thymidine. A rather precise localization may be achieved with labeled nucleoside because of its specific incorporation into DNA and the short range of tritium beta particles(1,2). The exogenous precursor is apparently available only for a brief period after administration, which is an added advantage. Neutrophilic leukocytes are part of a highly labile renewal system and the kinetics of their development *in vivo* can be determined from temporal changes in degree of labeling and distribution of the various cells in developmental sequence. The present investigations were initiated to establish the neutrophil life cycle in the normal steady state as a basis for evaluation of various perturbations of the neutrophil system including those induced by irradiation.

Methods. Thus far, 2 normal dogs (1 male and 1 female beagle, 1.5 and 4 yrs. old respectively) have been studied after intravenous injection of tritiated thymidine in dosage of 100 μc/kg body weight. The thymidine, labeled on the pyrimidine moiety, was radiochemically pure as judged by chromatographic and spectrophotometric analyses. Specific activity was 390 mc/mmole.† Bone marrow and peripheral blood studies were carried out at frequent intervals during the first 2 days, and daily thereafter for 2 weeks. Marrow samples (0.8 ml) were aspirated from femur or iliac crest into equal volume of heparinized plasma. The procedure was performed under local anesthesia in trained animals. Peripheral blood studies, which in-

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