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Studies on Gas Exchange and Acid-base Balance in the Tissue by Means of the Subcutaneous Liquid Pocket with Fluorocarbon Emulsion

Yasuko KODAKA and Yasuyo OHTA

Department of Physiology, School of Medicine, Tokai University

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A subcutaneous liquid pocket with fluorocarbon emulsion has been used as a new means to assess acid-base balance and gas exchange in the tissue. The pocket contents of rats under ambient air breathing showed a pO_2 of 45.8±3.3 Torr, pCO_2 of 49.0±1.3 Torr, pH of 7.374±0.052 and HCO_3^- of 17.1±2.2 mEq/L. Changes in the above parameters were studied under hypoxic, hypercapneic and asphyxic environments and comparied in part with those in arterial blood. Respiratory alkalosis under hypoxia and respiratory acidosis with some improvement in oxygenation in the tissues were observed. Acetazolamide given intraperitoneally under asphyxic conditions not only induced metabolic acidosis, but also caused a deterioration in tissue hypoxia, which may limit the therapeutic use of acetazolamide in respiratory acidosis.

(Key Words: Fluorocarbon, Liquid pocket, Tissue gas exchange, Tissue acid-base balance, Acetazolamide)

Since we are obliged to deduce changes in gas exchange and acid-base balance in the tissues indirectedly from data obtained from perfusing blood and/or expired gas, it is mandatory to establish ways of direct access to these physiological or pathophysiological states in the tissues. There are no comprehensive studies on acid-base regulation in the tissues except for those in cerebrospinal fluids in relation to chemical regulation of respiration (3,21).

Concerning tissue gas exchange, gas equilibration methods using the body cavities as *in vivo* tonometers were the first attempt to estimate tissue gas tensions (4,5). A gas mixture injected into the body cavity comes into equilibration with the surrounding tissues and yields oxygen and carbon dioxide tensions representing those in the tissue. The body lymph, gall bladder, urinary bladder and cerebrospinal fluids were also utilized as liquid pockets (1,2) in which the contents were expected to represent gas tensions of the surrounding tissues after equilibration.

Subcutaneous gas pockets, which were artificially made under the skin by introducing air percutaneously, were used in an attempt to estimate tissue gas tensions for both experimental and clinical purposes (10,25). These gas equilibration methods, however, did not offer any

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Correspondence to: Yasuyo OHTA, M. D. Department of Physiology, School of Medicine, Tokai University, Bohseidai, Isehara, Kanagawa 259-11, Japan

information on acid-base parameters except for carbon dioxide tension, and other ways to analyze venous blood reflux from a certain tissue or organ can not be utilized for technical and physiological reasons.

The present study is aimed at establishing a means of access to tissue acid-base balance in combination with tissue gas exchange, and to evaluate changes in these states under some experimentally induced conditions in animals.

METHODS AND MATERIALS

Male Wistar-strain rats weighing approximately 200 gm were used as experimental animals. The subcutaneous gas pockets were prepared by injecting air subcutaneously on the back of the neck, as reported previously (19, 20). After oxygen and carbon dioxide tensions (abbreviated as pO_2 and pCO_2, respectively) in the pockets reach a quasi-equilibrium, i.e., a state of constant composition (23), all the gas in the pockets was withdrawn and replaced with approximately 20 ml of fluorocarbon emulsion. The fluorocarbon liquid pockets served as tissue tonometers *in vivo*.

Fluorocarbon (abbreviated as FC) emulsion was prepared by the following procedure. First, pluronic F-68 was dissolved in normal Ringer solution to make a 10% surface active solution. The solution was filtered twice through a millipore filter with 8 micra pores and 20 vol% of FC-43 (Minnesota Mining and Manufacturing Company) was added to the pluronic-Ringer solution. The mixture was emulsified by means of a Branson Sonifier Cell Disruptor (Model B-12) for 6 minutes at an energy output of between 95 and 105 watts, while holding the step horn of the sonifier and the mixture in the iced-water bath.

The measurement of solubilities of O_2 and CO_2 in 20% FC emulsion was performed by equilibrating the emulsion with a known gas mixture by means of a Farhi duty tonometer (9) and by analyzing O_2 and CO_2 contents by the Van Slyke-Neill manometric apparatus (27). The Bunsen solubility coefficients were calculated according to Henry's law.

The first observations were made over the time courses of changes in pH, pO_2 and pCO_2 of the FC emulsion in the pockets for 6 hours immediately after the replacement of the pockets gases with FC emulsion.

The second experiments were conducted by having the experimental animals exposed to various gas atmospheres, i.e., hypoxic (10% O_2 in N_2), hypercapneic (8% CO_2 and 20% O_2 in N_2), asphyxic (10% O_2 and 8% CO_2 in N_2) and normoxic (20% O_2 in N_2) conditions, for 4-hours, with or without intraperitoneal administration of acetazolamide in a dose of 50 mg/kg of body weight.

Liquid samples were obtained from the pockets at certain time intervals and analyzed for pH, pCO_2 and pO_2. Arterial blood samples were obtained from the abdominal aorta through an abdominal incision under light pentbarbital sodium anesthesia and were analyzed for pH, pCO_2 and pO_2.

All of the gas samples were analyzed with a Scholander Micro Gas analyzer, and pH, pCO_2 and pO_2 of liquid and blood samples were

measured with an I. L. Meter, Model 213. Bicarbonate (abbreviated as HCO_3^-) concentration in the blood was calculated by means of the Henderson-Hasselbalth equation using numerical values of 6.10 for pK' and 0.03017 ml/100ml/Torr (12) for the solubility coefficient of CO_2. HCO_3^- concentrations in FC emulsion and those sampled from the liquid pockets were computed in terms of the Henderson-Hasselbalch equation applying the values of pH and pCO_2 obtained and a pK' of 6.484 estimated from the changes in pH, pCO_2 and CO_2 contents in tonometered FC emulsion with a known CO_2 gas mixture.

RESULTS

FC emulsion prepared ultrasonically from 20 vol% FC-43 and pluronic Ringer solution has particles with diameters between 1 and 12 micra, and no separation of the emulsion into the constituent liquid phases was observed for more than 3 days. The FC emulsion, when it was injected directly under the skin to form a subcutaneous liquid pocket, was absorbed rapidly and did not meet the requirements of these studies. However, when the FC emulsion was introduced into the established pocket, it remained for sufficiently long periods to accomplish the experiments.

The solubility coefficient for CO_2 in a 20% FC emulsion at 37°C was 0.7557±0.0187 ml/ml/760 Torr. The estimated pK' value for the HCO_3^- -H_2CO_3 system in the FC emulsion was 7.484±0.050.

The pO_2 and pCO_2 values of the pocket gas at a state of constant composition were 36.2±0.5 Torr and 45.7±0.7 Torr, respectively.

In Fig. 1 and Fig. 2, the time courses of changes in pO_2, pCO_2, pH and HCO_3^- in the pocket emulsion while breathing air are shown. The pO_2 and pCO_2 values showed relatively rapid changes during the initial 15 minutes and gradually reached equilibrated values at 90 to 120 minutes after introduction of the FC emulsion. The pH and HCO_3^- in the liquid pockets showed rather gradual changes and reached a quasi-equilibrium at

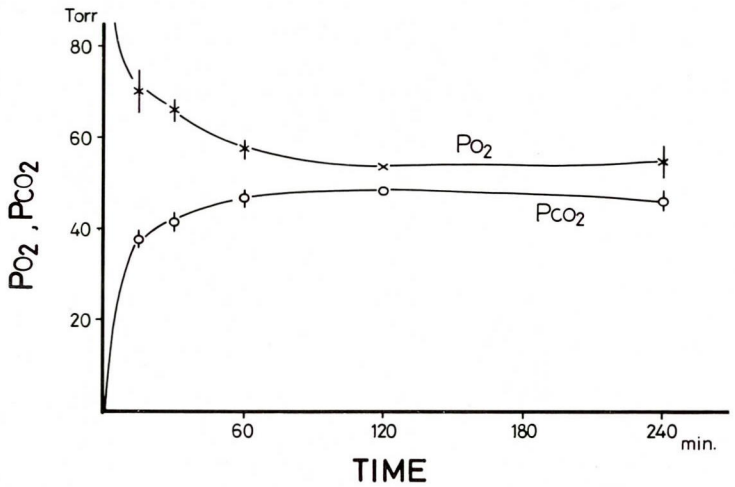

Fig. 1 Equilibration of pO_2 and pCO_2 in the liquid pockets after introduction of FC-emulsion. Bars indicate ± 1 S.E.

120 minutes after the FC emulsion injection, and no significant changes were seen until 360 minutes later. The mean values of pH, pCO_2, pO_2 and HCO_3^- of the 9 liquid pockets at equilibrium while breathing air were

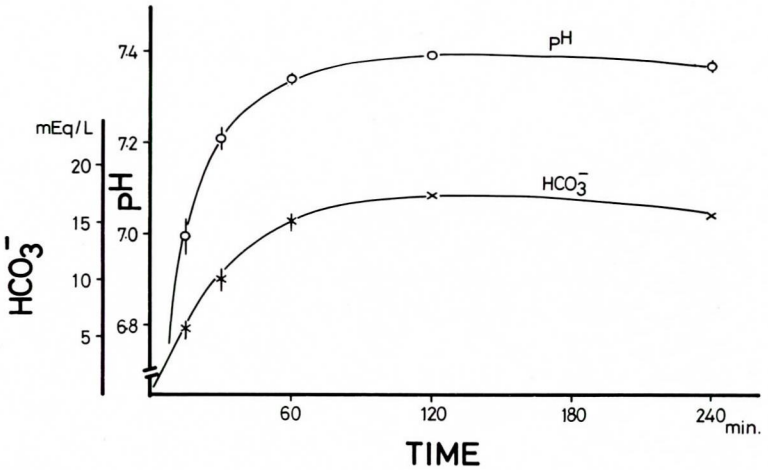

Fig. 2 Equilibration of pH and HCO_3^- in the liquid pockets after introduction of FC-emulsion. Bars indicate ± 1 S.E.

7.374±0.023, 49.0±0.6 Torr, 45.8±1.5 Torr and 17.1±1.0 mEq/L, respectively.

The pH, pCO_2, pO_2 and HCO_3^- values in arterial blood of the nine rats with subcutaneous liquid pockets were 7.403±0.013, 29.4±0.9 Torr, 94.0±2.2 Torr and 17.1±1.0 mEq/L, respectively, under air breathing.

Fig. 3 shows changes in pH of the pocket liquids under various experimental conditions. Under hypercapneic conditions, the pH decreased progressively for the first 120 min after exposure to CO_2. During further exposure from 120 min to 240 min, pH showed consistently low but rather constant values. Administration of acetazolamide under hypercapnia caused a more conspicuous lowering of the pH. Upon exposure to hypoxia, pH rose moderately for 120 min after exposure.

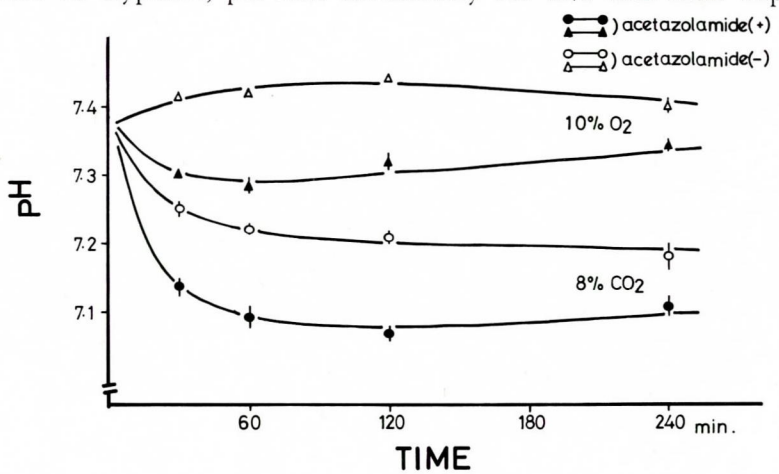

Fig. 3 Changes in pH in the liquid pockets under hypoxia and hypercapnia, and the effects of acetazolamide administration on pH. Bars indicate ± 1 S.E. ▲——▲ and ●——● indicate acetazolamide treatments for hypoxia and hypercapnia, respectively.

However, rats treated with acetazolamide un der hypoxia had slightly lower pH values in the pocket liquids than those in the control group.

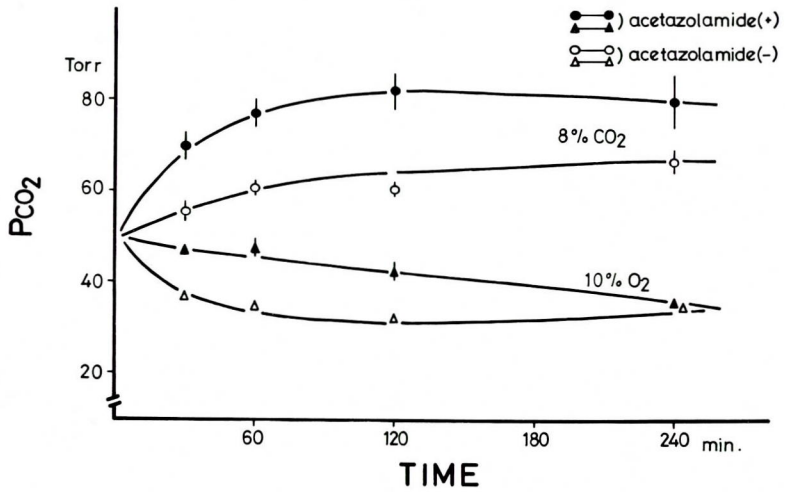

Fig. 4 Changes in pCO_2 in the liquid pockets under hypoxia and hypercapnia, and the effects of acetazolamide administration on pCO_2. For the symbols, see the legend of Fig. 3.

As seen Fig. 4 in contrast to Fig. 3, changes in pCO_2 in the pockets represent mirror images of those in pH.

The pO_2 in the pockets was consistently low in the hypoxic group, but in hypercapnia, it increased steadily for 120 min after exposure to CO_2 and then started falling slightly. In both hypoxic and hypercapneic

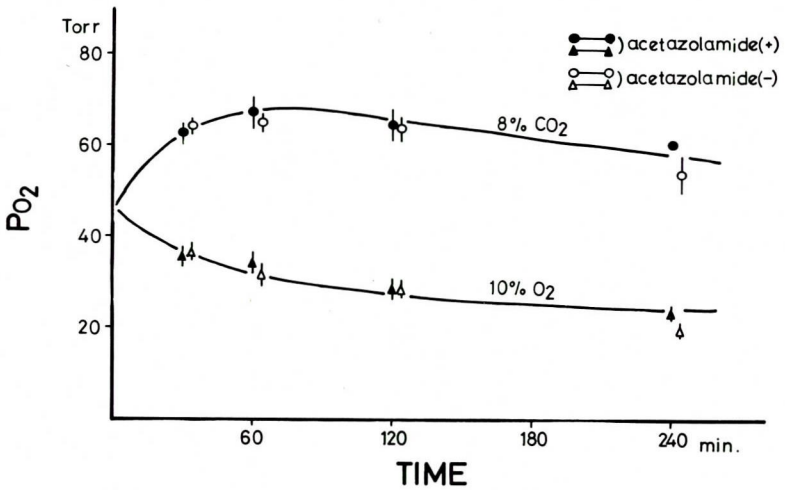

Fig. 5 Changes in pO_2 in the liquid pockets under hypoxia and hypercapnia, and the effects of acetazolamide treatment on pO_2. See the legend of Fig. 3 for the symbols.

groups, acetazolamide given intraperitoneally had no significant effect on the tissue pO_2, and therefore, Fig. 5 has only two curves.

The HCO_3^- in the pockets showed an initial decrease for the first 60 min after exposure to hypercapneic environment, and then a tendency for recovery to the control value. In the hypoxic group of rats, HCO_3^-

decreased rapidly and stayed at the lower level for 240 min. Acetazolamide administration consistently enhanced those decreases in HCO_3^- concentration in the pocket liquids.

Table 1 summarizes data of pH, pCO_2, pO_2 and HCO_3^- obtained after 60 minutes of exposure to each of the experimental conditions, i.e., hypoxia, hypoxia with acetazolamide treatment, hypercapnia and hypercapnia with acetazolamidae treatment.

Table 1 Changes in pH, pCO_2, pO_2 and HCO_3^- in the liquid pockets of rats under either hypoxia or hypercapnia, and the effects of acetazolamide treatment on these parameters. Samples were taken after 60 min of exposure to the experimental condition.

	Hypoxia		Hypercapnia	
	acetazolamide (−)	acetazolamide (+)	acetazolamide (−)	acetazolamide (+)
	(n=8)	(n=7)	(n=7)	(n=6)
pH	7.420 ± 0.008	7.284 ± 0.011	7.221 ± 0.007	7.092 ± 0.016
pCO_2 (Torr)	34.8 ± 0.9	47.7 ± 1.9	60.8 ± 1.6	77.1 ± 3.1
pO_2 (Torr)	31.7 ± 2.5	34.3 ± 2.3	64.9 ± 1.9	67.2 ± 3.2
HCO_3^- (mEq/L)	13.4 ± 0.1	13.5 ± 0.3	14.9 ± 0.5	14.0 ± 0.2

Acetazolamide administration under air breathing had no significant effect on the tissue pO_2, while a moderate fall in pH and a rise in pCO_2 were observed.

Under asphyxic conditions in which the animals breathed a gas mixture of 10% O_2, 8% CO_2 and the rest N_2, changes in acid-base parameters and in gas tensions were more striking in some aspects compared to those in hypoxia and/or hypercapnia (Figs. 6, 7 and 8). A marked fall pH and a rise of pCO_2 in both arterial blood and the pocket liquids were observed, and they were decidedly enhanced by acetazolamide administration. However, gradual recoveries of arterial pH and

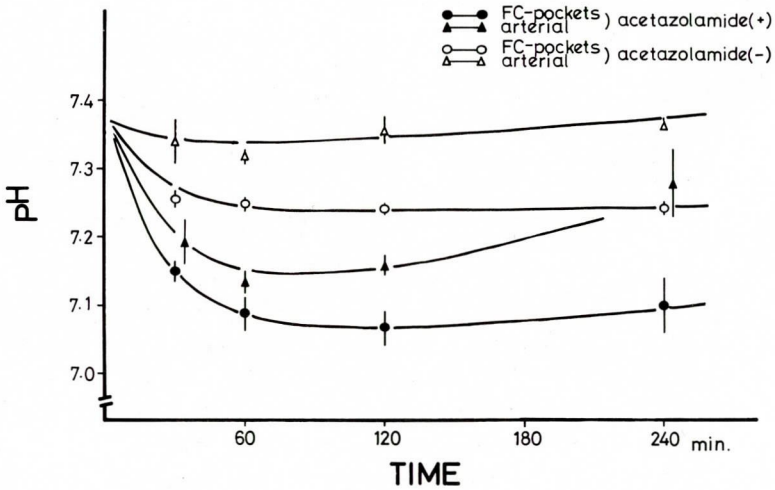

Fig. 6 Changes in pH in the liquid pockets and in arterial blood of rats under asphyxia. Bars indicate ± 1 S.E.

arterial pCO_2 to the control level were seen 120 min after the beginning of exposure to asphyxia when the animals were treated with acetazolamide. The pO_2 values of arterial blood under asphyxia were slightly higher than those of the control group. However, when acetazolamide was given, pO_2 increased rapidly for the first 60 min after exposure and then gradually returned to the control level. However, pO_2 in the pockets showed a slight decrease throughout the observation period, and acetazolamide treatment under asphyxia showed a consistent effect in lowering the pocket pO_2 (Fig. 8).

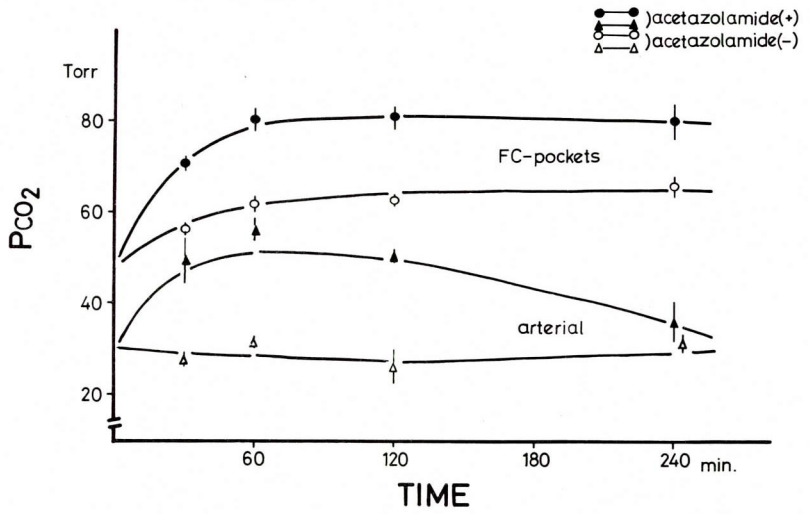

Fig. 7 Effects of acetazolamide treatment on pCO_2 in the liquid pockets and in arterial blood of rats under asphyxic environment. Bars indicate ± 1 S.E.

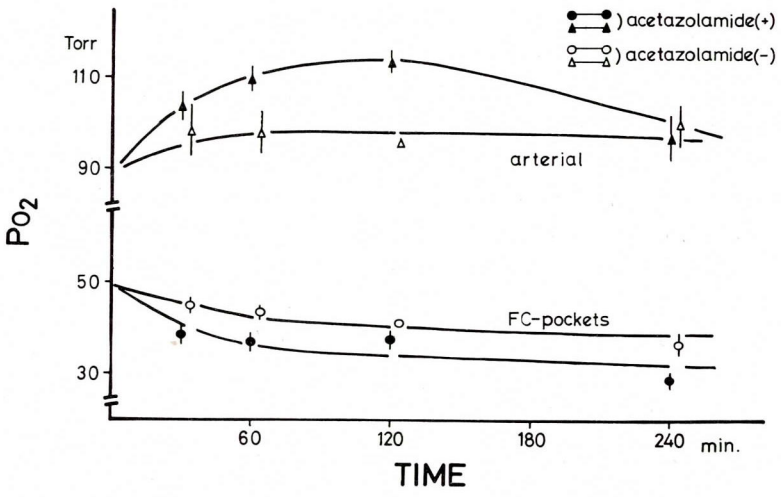

Fig. 8 Effects of acetazolamide treatment on pO_2 in the liquid pockets and in arterial blood on rats under asphyxic environment. Bars indicate ± 1 S.E.

Changes in HCO_3^- concetration in both arterial blood and the pocket liquids showed a pattern of initial decrease and recovery to the control level after 240 min of exposure. Acetazolamide interfered with this recovery of the pocket HCO_3^-, and kept the decreased values for the following period. However, arterial HCO_3^- of the acetazolamide-treated rats showed a slight increase for the first 60 min after exposure to asphyxia and returned to the control level after 240 min.

Effects of asphyxia and acetazomamide administration on pH, pCO_2, pO_2 and HCO_3^- in both arterial blood and the pocket liquids are summarized in Table 2.

Table 2 Changes in pH, pCO_2, pO_2 and HCO_3^- in the liquid pockets and in arterial blood of rats under asphyxic environment, and effects of acetazolamide administration. Data were obtained 60 min after exposure to asphyxia.

	Pockets		Arterial blood	
	acetazolamide (−) (n=10)	acetazolamide (+) (n=9)	acetazolamide (−) (n=4)	acetazolamide (+) (n=4)
pH	7.248 ± 0.010	7.089 ±0.025	7.318 ±0.011	7.133 ± 0.017
pCO_2 (Torr)	61.7 ±1.7	80.1 ± 4.3	31.5 ± 1.3	56.3 ± 2.5
pO_2 (Torr)	43.3 ± 1.6	36.8 ± 2.2	98.0 ± 4.7	107.9 ± 2.7
HCO_3^- (mEq/L)	16.0 ± 0.4	14.1 ± 0.4	15.7 ± 1.0	18.3 ± 1.0

DISCUSSION

A fluorocarbon emulsion was prepared according to Clark's method (8) with some modifications. Since the emulsion has very high acidity (for example, the FC emulsion in the present study has a pH of 3.30), THAM (tris-hydroxymethyl-amino-methane) or some other alkalinizing agents have been added to the FC emulsion. However, we did not use these buffer solutions because buffer action of these agents would make changes in pH and other parameters obscure in the present study. Hence, introduction of FC emulsion into the subcutaneous pockets may give rise to some tissue reactions due to strong acidity. However, we started exposing the animals to the various experimental conditions 120 min after the introduction of FC emulsion during which pH, pO_2 and pO_2 reached a quasi-equilibrium.

The solubility coefficient obtained in the present study for CO_2 in the FC emulsion is slightly higher than that calculated from solubilities in pure fluorocarbon and in saline (22). The difference may be derived from the fact that 10% pluronic solution was used in the emulsion, or from the difference in measuring methods (15, 26).

In relation to CO_2 solubility in FC emulsion, it must be kept in mind that, in computing pK' for the CO_2-HCO_3^- system in the pocket liquids, we used a value of CO_2 solubility obtained from the analyses of pure FC emulsion, not of the mixture of FC emulsion and tissue fluids. This results in the possibility that the application of pK' thus obtained to the calculation of HCO_3^- in the pocket liquids may be very misleading in cases

where a large amount of transudation or water exchange between the surrounding tissues and the pocket contents occur. However, the volume of the liquid pockets decreased gradually and the liquid samples obtained during the course of the present study still kept the original appearance of the FC emulsion.

The validity of using liquid pockets in assessing gas exchange and acid-base balance in the tissues may be questioned in some other aspects. The weight of liquid in the pockets, for instance, may disturb blood perfusion by compressing the capillary nets of the pockets. However, this can be explained by the fact that pO_2 and pCO_2 in the pockets showed similar values to those previously reported (6, 7). The equilibration of substances between the pocket content and surrounding tissues should be fast enough compared to the time course of the experiment. Our present observations showed that pO_2 and pCO_2 equilibrations were much faster than those in the gas pockets, and 120 minutes should allow pH and HCO_3^- sufficient time for equilibration. One of the most serious questions can be raised against dynamic functions of HCO_3^- exchange between the pocket liquid and the tissues, and the validity of using the above-mentioned pK' value for estimating HCO_3^- concentration in the liquid pockets. We know little about these points and the further studies are required.

An elevation of pH under hypoxic conditions can be explained as a result of CO_2 depletion, and metabolic acidosis induced by acetazolamide counteracts this hypoxic effect on tissue pH. These observations are in line with the reports on the effects of acetazolamide in preventing alkalization of blood under hypoxia (13, 28).

A marked fall of pH and significant elevation of pCO_2 in the liquid pockets under hypercapnia is reasonable because of 8% CO_2 breathing, and the elevation of pO_2 might be explained as a result of CO_2-induced hyperventilation and improvement of blood circulation in the tissues. The results obtained suggest that acetazolamide worsened not only pH but also CO_2 accumulation in the tissues when it was given under hypercapneic conditions.

Changes in pH, pCO_2 and HCO_3^- under asphyxia were similar to those under hypercapnia. However, acetazolamide administration resulted in a further decrease in pO_2 in the tissues, while that in arterial blood remained unchanged. Since arterial blood was sampled while breating ambient air under anesthesia, we can not depend on the absolute values of blood gas data obtained in the present study. In the case of arterial pO_2 for example, it exceeds the maximal value which can be attained under 10% O_2 breathing. Nevertheless, we may deduce that acetazolamide induced CO_2 retention in the blood as well as the tissues, and it was not due to aleveolar hyperventilation.

The dose of acetazolamide used in the present study has been reported to block the activity of carbonic anhydrase in the body completely, and the half life of acetazolamide given orally to rats is estimated to be 65 minutes (14). In such a situation, a rapid chemical equilibration of $CO_2 + H_2O \rightleftharpoons H_2CO_3$ can not be attained, and the application of the Henderson-Hasselbalch equation is misleading (16). This

might be a reason for the unexpectedly high HCO_3^- concentration in arterial blood under asphyxia.

The accumulation of CO_2 in arterial blood should result in a decrease in O_2 loading, and that in the tissue should reduce O_2 unloading to the tissue.

If we can assume that arterial O_2 content remained unchanged when acetazolamide was given under asphyxia, the arterial-pocket liquid O_2 content difference was assumed to have increased by acetazolamide administration. Unless acetazolamide decreases local blood flow (17), the increase in O_2 content difference should indicate an increase in O_2 uptake in the tissue, probably due to hyperventilation. The elevation of pO_2 in the pocket by acetazolamide administration under asphyxia might result from increased CO_2 production as well as from impaired CO_2 transport.

It can be concluded that the therapeutic use of carbonic anhydrase inhibitors in respiratory acidosis (11, 18, 24) may be errorneous because they induce not only metabolic acidosis but also tissue hypoxia.

REFERENCES

1) Bergofsky EH, Jacobson and Fishman AP: The use of lymph for the measurement of gas tensions in interstitial fluid and tissues. J. Clin. Invest. 41 (11): 1971, 1962.
2) Bergofsky EH: Determination of tissue O_2 tensions by hollow visceral tonometers: Effect of breathing enriched O_2 mixture. J. Clin. Invest. 43 (2): 193, 1964.
3) Bradley RD et al.: Rate of change of CSF pCO_2 and $[HCO_3^-]$ in acid-base distribution in man. In: Cerebrospinal fluid and the regulation of ventilation. ed. by Brooks CC, FF Kao and Lloyd BB p281, Oxford, 1965.
4) Cambpell JA: The carbon dioxide partial pressure in body cavities and tissue spaces under various conditions. J. Physiol. 56: 273, 1923.
5) Campbell JA: Changes in the tensions of CO_2 and O_2 in gases injected under the skin and into the abdominal cavity. J. Physiol. 59: 1, 1924.
6) Campbell JA: The influence of O_2-tension in the inspired air upon the O_2 tension in the tissue. J. Physiol. 60: 20, 1925.
7) Campbell JA: Tissue oxygen tension and hemoglobin. J. Physiol. 65: 255, 1928.
8) Clark LC, S Kaplan and Becattini F: The physiology of synthetic blood. J. Thor. Cardiovas. Surg. 60 (6): 757, 1970.
9) Farhi LE: Continuous duty tonometer system. J. Appl. Physiol. 20: 1098, 1965.
10) Forgacs P: Subcutaneous gas equilibration in clinical practice. Thorax. 15: 37, 1960.
11) Galdston M: Respiration and renal effects of carbonic anhydrase inhibitor (DIAMOX) on acid-base balance in normal man and in patients with respiratory acidosis. Am. J. Med. 16: 516, 1955.
12) Jones ES, BG Maegraith and Sculthorpe HH: Pathological process indisease II-Blood of the albino rats: Approximate physico-chemical description. Ann. Trop. Med. Parasitol. 44: 168, 1956.
13) Mani KV and Weinstein SA: Effect of carbonic anhydrase inhibitor on blood gas and acid-base balance during hypoxia. Bull. Johns. Hopkins. Hosp. 119:331, 1966.
14) Maren TH: Carbonic anhydrase: Chemistry, physiology and inhibition. Phsiological Rev. 47: 595, 1967.
15) Mears WH and Beavers RL: Solubility of oxygen and carbon dioxide in a per-

fluorinated diether. Fed. Proc. 29 (5): 1819, 1970.
16) Mithoefer JC, PW Mayer and Stocks J: Effect of carbonic anhydrase inhibition on the cerebral circulation of the anesthetized dog. Fed. Proc. 16: 382, 1957.
17) Mithoefer JC and Davis JS: Inhibition of carbonic anhydrase: Effect on tissue gas tensions in the rats. Proc. Soc. Exp. Biol. Med. 98: 797, 1958.
18) Nadell J: The effects of the carbonic anhydrase inhibitor "6063" on electrolytes and acid-base balance in two normal subjects and two patients with respiratory acidosis. J. Clin. Invest. 32: 622, 1953.
19) Othta Y et al.: Gas exchange in the subcutaneous gas pocket (1) Tissue gas tensions (in Japanese). Kokyu to Junkan 14: 33, 1966.
20) Ohta Y et al.: Tissue gas tensions of the rat's subcutaneous gas pockets and the oxygen intoxication. In: Proc. 4th International Congress on Hyperbaric Medicine, Tokyo, 1970.
21) Palvin EG and Harnbein TF: Distribution of H^+ and HCO_3^- between CSF and blood during respiratory acidosis in dogs. Am. J. Physiol. 228 (4):.1145, 1975.
22) Peterson RE: Measurement of the solubility of gases in oils. Fed. Proc. 29 (5): 1714, 1970.
23) Piiper J, Canfield RE and Rahn H: Absorption of various inert gases from subcutaneous gas pockets in rats. J. Appl. Physiol. 17: 268, 1962.
24) Riley RL: The work of breathing and its relation to respiratory acidosis. Ann. Int. Med. 41: 172, 1954.
25) Seevers MH: O_2 and CO_2 tensions in the subcutaneous tissues of normal subjects. Am. J. Physiol. 115: 38, 1936.
26) Technical Information Bulletin, 3M Brand Inert Fluorochemical Liquids 3M Co., Chemical Division, 1965.
27) Van Slyke DD and Neill JM: Determination of gases in blood and other solutions by vacuum extraction and manometric measurement. J. Biol. Chem. 61: 523, 1924.
28) Weinstein SA and Riley RL: The effects of hypoxic and renal carbonic anhydrase inhibition upon learned escape from CO_2. Fed. Proc. 24: 272, 1965.