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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stimulated either directly or indirectly, it is evident that this is not due to a hemodynamic effect(2). On the contrary, it is possible that adrenergic blocking agents inhibit muscular performance directly by interfering with ionic movements across the cell membrane.

Pitressin, even in small doses, has been reported to shift water and sodium out of the extracellular space even in presence of adrenergic blockade(3), and into the cells of at least one tissue, the aorta(4). It has also been shown to be effective in partially restoring work performance in the adrenalectomized-hypophysectomized rat(5). On this basis, preliminary experiments were carried out which showed that a single dose of intravenous Pitressin could restore running ability almost completely in rats immobilized by adrenergic blockade. The present experiments were designed to study this response in detail.

Methods. Adult male albino rats of an inbred Wistar strain, 6 months of age and weighing 270-310 g, were used. The rats were anesthetized with pentobarbital (3.33 mg/100 g body weight) and phenobarbitone sodium (6 mg/100 g) administered intraperitoneally and subcutaneously respectively. The kidneys were then decapsulated and the pedicle ligated. An inulin solution, approximately 25% w/v was injected into the tail vein in a dose of 0.5 ml/100 g body weight. The absolute amount of inulin injected was accurately determined in each case by analysis of an aliquot taken from the injection syringe. After 3 hours of equilibration, polyethylene catheters were implanted into the right femoral artery and vein, an initial blood sample (0.9 ml) was taken from the artery and a control specimen excised from the right gastrocnemius muscle. Dibenzylene† at a dose of 250 μg/100 g body weight in 0.2 ml of saline was introduced into the femoral vein and the animal at once prepared and mounted for kymographic recording of contractions of the left gastrocnemius activated by stimulation of the cut end of the sciatic nerve. The gastrocnemius muscle was then stimulated at a rate of 20 twitches/second for 20 minutes. A dose of 80 mU of Pitressin in 0.2 of saline was given intravenously when the effect of Dibenzylene was close to its peak and muscular contractions had not entirely ceased. Control animals were sham operated and received comparable volumes of saline. After 20 minutes stimulation a second blood sample was taken through the femoral cannula and the left gastrocnemius muscle was quickly excised.

Intracellular water, sodium and potassium determinations were carried out, with minor modification, by the methods of Ledingham 6) as follows: muscle samples were blotted with Whatman No. 42 filter paper, cut into several pieces and weighed at once, then either dried to a constant weight at 105°C for determination of water content or used for determination of muscle inulin according to the method of Ross and Mokotoff(7). Muscle samples used for estimation of total water content were then extracted free of fat by the method of Hastings and Eichelberger(8) and also used for sodium and potassium determinations. Dried samples were wet ashed in 1.0 ml of concentrated nitric acid, diluted and analyzed by flame photometry. Results are expressed as meq/l intracellular fluid. Blood was analyzed for glucose by the method of Nelson(9) with the copper reagent as modified by Somogyi(10), and for lactic acid by the method of Barker and Summerson (11). Plasma was analyzed for inulin by the method of Higashi and Peters(12) and for sodium and potassium by flame photometry. The volume distribution of inulin was taken as a measure of extracellular water. The amount of intracellular water per kg of fat-free muscle was estimated by subtracting extracellular water/kg fresh fat-free muscle from the measured water content of the whole muscle. Intracellular sodium and potassium were calculated by deducting from the total amount of tissue ions the amount contained in a known volume of extracellular water.

Thirty-five rats were divided into 4 groups. Group 1 (7 rats) served as untreated, sham operated control; group 2 (8 rats) was given Dibenzylene (250 μg/100 g intravenously); group 3 (8 rats) was given Pitressin (80 mU

† Dibenzylene was supplied through the courtesy of Smith, Kline and French.

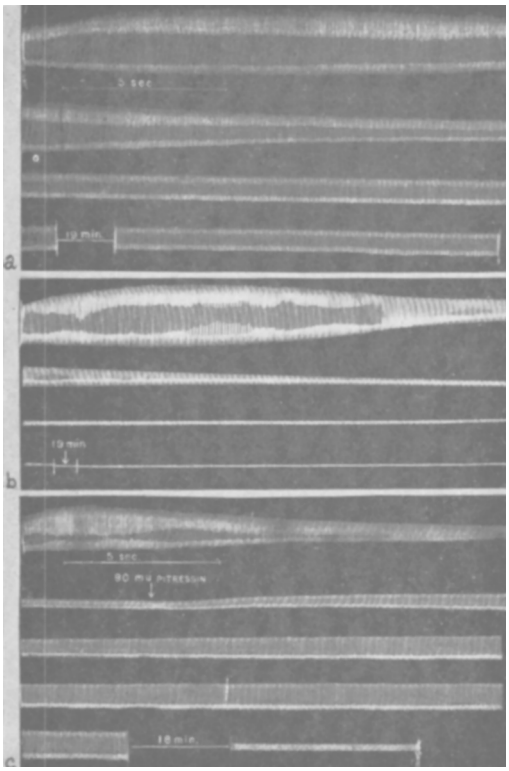

FIG. 1. Responses of adult rat gastrocnemius *in situ* to sciatic nerve stimulation, 20 twitches/sec. (5-6 V, 0.2 msec. duration) for 20 min. (a) Normal control, (b) following intrav. Dibenzylene 250 μg/100 g, (c) following intrav. Dibenzylene countered with Pitressin.

intravenously) and group 4 (12 rats) both Dibenzylene and Pitressin.

Results. Effect of Pitressin on Contractility of Dibenzylene Blocked Gastrocnemius in situ. When the gastrocnemius was stimulated indirectly at 20/second the height of contraction, except for an initial unstable period of less than half a minute, remained essentially unchanged throughout the 20 minutes of stimulation (Fig. 1a). In sharp contrast, the height of muscle contraction decreased markedly after Dibenzylene so that within a few minutes only a scarcely discernible wave remained to mark each stimulus (Fig. 1b). When 80 mU of Pitressin was administered intravenously before the contractions had entirely diminished, Dibenzylene suppression was eliminated for a period of 12-16 minutes before the effect wore off (Fig. 1c). These effects were entirely reproducible.

Effect of Pitressin on Ion Exchanges in Dibenzylene Blocked Gastrocnemius in situ. The essential changes in intracellular and extracellular water, sodium and potassium corresponding to the changes in contractility just described are shown in Fig. 2.

In the control group, intracellular water and sodium increased while potassium decreased following the period of work as previously described(2). Dibenzylene hindered the cationic exchange as it suppressed contractility but did not much affect the water shift. Pitressin was effective in restoring the transmembrane movements of sodium and potassium in the Dibenzylene treated animal as it restored contractility. Pitressin alone did not affect the sodium, potassium or water shift produced by exercise.

Discussion. Ingle and Li reported that while an adrenal cortical extract could not entirely correct the work deficit of adrenalectomized-hypophysectomized rats such an extract given together with a very small amount of vasopressin could normalize work performance(5). We later reported that Pitressin produced a movement of sodium and water out of and potassium into the extracellular space(3). Daniel *et al.* showed this to mirror, in part, shifts into and out of cells in the aorta wall(4) and we have now broadened this to include skeletal muscle as well (in preparation). The present experiments suggest that these observations are all interdependent.

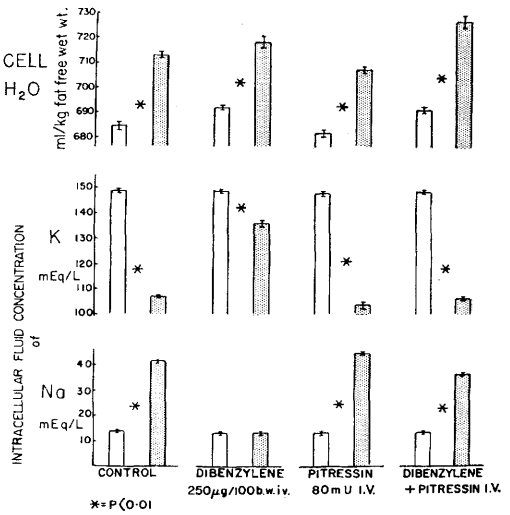

FIG. 2. Effect of stimulation of gastrocnemius *in situ* on intracel. water, Na and K in intact, Dibenzylene and Pitressin treated adult rats.

It is generally held that the action potential which triggers the contractile process depends on a transmembrane ionic exchange characterized first by an inward movement of sodium followed rapidly by an efflux of potassium (13). Adrenergic blocking agents, in relatively high doses, have been shown to interfere with the transmembrane exchange of ions in resting muscle incubated *in vitro* and this is accompanied by an impaired contractility measured *in vivo, in situ* or *in vitro*(2). Pitressin, which can shift sodium and potassium in the continuing presence of such an adrenergic blockade, restores contractility to the rat gastrocnemius during the limited period of its effectiveness. Perhaps the observation of Ingle and Li rests on the same ability of Pitressin to transfer sodium and potassium (with water) across the cell membrane.

Summary. Intracellular sodium concentration of stimulated rat gastrocnemius muscle increased 3-fold while potassium decreased by almost one-third after 20 minutes of stimulation (20/sec.). Dibenzylene abolished these changes and strikingly reduced the amplitude of muscular contraction. Pitressin (80 mU intravenously) in Dibenzylene treated rats restored both the cationic shifts and muscular contractions during its period of effectiveness. We suggest that the ability of Pitressin to restore work performance is brought about by a direct action on cationic exchanges across the muscle membrane.

1. Sréter, F. A., Friedman, S. M., *Am. J. Physiol.,* 1959, v197, 478.

2. ———, *J. Pharmacol. and Exp. Therap.,* in press.

3. Friedman, S. M., Friedman, C. L., Nakashima, M., *Nature,* 1957, v180, 194.

4. Daniel, E. E., Dodd, A., Hunt, J., *Arch. Internat. Pharmacodyn.,* 1959, v119, 43.

5. Ingle, D. J., Li, C. H., *Endocrinology,* 1955, v57, 383.

6. Ledingham, J. M., *Clin. Sci.,* 1954, v13, 535.

7. Ross, G., Mokotoff, R., *J. Biol. Chem.,* 1951, v190, 659.

8. Hastings, A. B., Eichelberger, L., *ibid.,* 1937, v117, 73.

9. Nelson, N., *ibid.,* 1944, v153, 375.

10. Somogyi, M., *ibid.,* 1945, v160, 61.

11. Barker, S. B., Summerson, W. H., *ibid.,* 1941, v138, 535.

12. Higashi, A., Peters, L., *J. Lab. and Clin. Med.,* 1950, v35, 475.

13. Hodgkin, A. L., *Biol. Rev.,* 1951, v26, 339.

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Immunologic Mechanism of Transmission of Experimental Glomerulonephritis in Parabiotic Rats.* (26221)

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We reported(1) that in patients with glomerulonephritis antibodies against human kidney could be detected by means of the very sensitive but also very delicate collodion technic. These findings were confirmed by Pfeiffer *et al.*(2) and by Brod(3) with the same technic and by Vorlaender(4) with a sensitive complement fixation technic. More recently Liu and McCrory(5) using the tanned red cell technic and the Ouchterlony gel-precipitation method have come to identical conclusions. On the other hand, Heyman(6) and Goodman(7) could not find such antibodies. All investigators who demonstrated such antibodies agreed with us that antibody titers were lowest or absent in the acute stage of glomerulonephritis and became higher and more consistent later in the disease. We assumed at first that in the acute stage the antibody was completely absorbed by the kidney which was supposed to act like

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