process on pH(10). It is conceivable that any potassium which might be bound in muscle at rest or in the "normal" state becomes free and diffuses out as a result of drug action. The idea that such a release occurs when contraction takes place is far from new(11). 3. *Change in muscle membrane permeability.* In the absence of contraction, this possibility, that the cell is simply made more permeable to potassium, is unlikely. The fact that muscle swelling was not altered by the drug in KR lends improbability to such a notion.

It is possible that more than one mechanism was operative here. If as much as 33% of muscle potassium is bound or non-diffusible (12), then it is possible that some of the 20% loss came from this fraction. Since contraction can be produced by the drug, the changes related to normal function were undoubtedly reflected in the results above (the usually-seen potassium loss, sodium and water gains, and associated permeability changes).

Summary. Mephenesin in concentrations up to 0.3% caused no change in the swelling resulting from immersion in high-potassium Ringers. Mephenesin at a concentration of 0.3%, but not at 0.1%, caused a large, reversible increase in swelling resulting from immersion in sodium-poor, hypotonic Ringers. This relatively high drug concentration also caused a 20% loss of muscle potassium both in regular Ringers and in hypotonic, sodium-poor Ringers. These changes are thought to be related to muscle contraction produced by Mephenesin. Confirming results of similar experiments by Fenn(7), the procedure of disturbing or handling muscles at intervals throughout an experiment was seen to result in muscle weight gain, a small sodium increase, and little alteration of potassium content; this process was intensified by Mephenesin.

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1. Stephen, C. R., and Chandy, J., *Can. Med. Assn. J.*, 1947, v57, 463.

2. Berger, F. M., *Brit. J. Pharm.*, 1947, v2, 241.

3. Goodman, J. W., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 500.

4. Fenn, W. O., Cobb, D. M., and Marsh, B. S., *Am. J. Physiol.*, 1934, v110, 261.

5. Fisher, R. B., and Subrahmanyan, V., *J. Physiol.*, 1939, v97, 233.

6. Shanes, A. M., *Science*, 1948, v107, 679.

7. Fenn, W. O., *J. Cell. Comp. Physiol.*, 1936, v9, 93.

8. Wright, J. L., PhD Thesis, University of Rochester, 1952.

9. Fenn, W. O., and Cobb, D. M., *Am. J. Physiol.*, 1936, v115, 345.

10. Erdös, T., and Snellman, O., *Biochem. Biophys. Acta*, 1948, v2, 642.

11. Fenn, W. O., *Physiol. Rev.*, 1940, v20, 377.

12. Stone, D., and Shapiro, S., *Am. J. Physiol.*, 1948, v155, 141.

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Effect of Air Ions on Succinoxidase Activity of the Rat Adrenal Gland.* (21223)

CHRISTEN B. NIELSEN† AND HAROLD A. HARPER.‡

From the Department of Biology, University of San Francisco, Calif.

The atmosphere contains at all times variable amounts of gaseous molecules that possess a negative or positive charge. The concentration of these "air ions" under normal conditions may vary between 400 and 2000 ion pairs/cc, but under certain conditions an excess of either positively or negatively charged ions may occur. The possible physiologic effect of air ions has interested a number of investigators. In a series of studies carried

* From thesis submitted by Christen B. Nielsen to Graduate Division of University of San Francisco, in partial fulfillment of requirements for degree of Master of Science.

† Wesix Research Fellow, 1952-54. Present address, 816 Cowper St., Palo Alto, Calif.

‡ Present address, University of California Medical Center, San Francisco.

out with human subjects exposed to ionized atmospheres containing an excess of positive or of negative ions(1,2), it was reported not only that physiologic effects were demonstrated but that differences were observed in the response to an excess of positive as compared to negative air ions. In the period intervening between these early experiments and the present, improved methods have been devised for the generation of air ions and their separation so as to produce an excess of ions of either positive or negative polarity. Skilling and Beckett(3) have recently described a method for the production and separation of positive and negative air ions. The ionization of the air is accomplished by alpha particles from radioactive polonium 210. Ion separation is then brought about by the use of a charged screen adjacent to the polonium emitter. In the electrostatic field thus produced, the ions oppositely charged to that of the screen are attracted toward the electrode and discharged. Those ions having the same charge as the screen are repelled and travel into the air to produce an excess of positive or negative ions, depending on the charge originally placed on the screen. This ionizing device has been used to produce controlled ion atmospheres in studies of the physiological effects of such atmospheres on experimental animals. Worden(4) maintained hamsters in ionized air at concentrations of approximately 1600/cc of one polarity and 200 of the opposite. He reported that the carbon dioxide capacity of the plasma was significantly increased in those animals kept in negatively ionized air as compared to those in positively ionized air or in a normal atmosphere. The blood pH was also elevated in the negatively ionized group(5). Blood samples for analysis were drawn from individual animals sacrificed at 24, 48, 72 or 96 hours after ionization had begun. The changes reported were observed at the end of the first (24-hour) period, as well as in each of the subsequent periods. Later work showed that the effects could be demonstrated after a one-hour exposure and, furthermore, that the plasma chloride concentrations varied inversely with the CO_2 capacity.

Several investigators have reported on evidence for increased activity of the adrenal cortex in animals exposed to ionized air. Holloway(6) investigated the changes in cholesterol and ascorbic acid which occurred in the adrenals of rats maintained for varying periods of time up to 72 days in positively or negatively ionized air. Rinfret and Wexler(7) using histological methods for studies of adrenal changes found that exposure to an atmosphere containing an excess of positive ions (2500-3000/cc) produced histological changes indicative of elaboration by the glands of both salt and carbohydrate-regulating corticoids. In this paper we are reporting the results of an investigation of the succinoxidase activity of homogenates from the adrenal glands of rats maintained in ionized air. McShan *et al.*(8) in examining the succinoxidase activity of homogenized endocrine tissues found that the Qo_2 of the adrenals was higher than that of any other endocrine tissue studied and they suggested that the succinoxidase content could be correlated with the activity of the gland.

Methods. Male Sprague-Dawley rats, 200-240 g in weight were used. For analyses of the succinoxidase activity of the adrenal glands, the animals were sacrificed by rapid decapitation. Both glands from a given animal were removed as quickly as possible, chilled, trimmed and homogenized for one minute in 2 ml of ice-cold triple distilled water using a Potter-Elvehjem all-glass homogenizer. The homogenate was diluted to 4 ml and stored in the freezing compartment of the refrigerator until used for assay (usually within less than one hour). The method of Schneider and Potter(9) was employed for the manometric assay of succinoxidase. Oxygen uptake served as a measure of the activity of succinoxidase in the homogenate. Nitrogen determinations were made on one-ml aliquots of the homogenate by a micro Kjeldahl method(10) and the oxygen uptake was then expressed as Qo_2, N, *i.e.*, μl oxygen uptake per mg tissue nitrogen per hour. Preliminary control experiments suggested that the succinoxidase activity of the adrenal was readily affected by a number of extraneous stimuli such as light, temperature, noise, increased activity within the animal room, frequent handling of

the animal, and the feeding and weighing schedule. Attempts were made to control these variables as closely as possible; the illumination in the animal room was confined to daylight from a light well; the temperature of the room was maintained at 80°F; an effort was made to select only those animals which seemed well adjusted to their surroundings and each test animal was isolated from the group for at least 4 days prior to a given experiment.

Results. In consideration of the diurnal variations in the activity of the animals, it was of interest to compare the succinoxidase content of the adrenal gland observed at periods of minimum and maximum activity. The following results were obtained in the course of experiments conducted during the summer season (July and August). The succinoxidase of the adrenal homogenates obtained from normal rats during a period of minimum activity (day) averaged 175 (±8.5)§; during a period of maximum activity (night), 226 (±19.3). The difference, which represents a 52% increase in activity, is statistically significant (P value less than 0.01). It is apparent from these data that the activity status of the animal exerts an important effect on the succinoxidase content of the adrenal gland of the rat. The change from minimum to maximum activity may occur in a very short period of time. Any sudden disturbance in the animal room or the repeated withdrawal of animals from a group in a single cage, for example, would alter the activity of the animals and cause a rise in succinoxidase to a maximum. In an attempt to minimize this experimental variable our studies were carried out during the period of maximum activity when slight disturbances within the animal room would be less likely to cause a further increase in the activity of the animal.

Ionization of the air in the animal cage was accomplished by the installation on the roof of a 7 x 15 x 10 inch metal cage of the tubular type ionizer described by Skilling and Beckett(3). Ionization of molecules in the air was produced by the alpha emissions from a polonium foil. The type of ionization which predominated was controlled by the charge placed on the outer metal tube of the ionizer. This charge was altered in accordance with the connection made to the appropriate terminal of a 300-volt B battery. Ion density was measured by the use of an ion collector in combination with a Beckman Ultrometer. The concentration of small ions (*i.e.*, ions with the highest mobility) in the air of the negatively ionized cages averaged 4500-6000 negative ions/cc and 300-400 positive; in the positively ionized cages, 12000-17000 positive ions/cc and 200-250 negative. The average mobilities of the negatively charged ions are higher than the positive. As a result, the negative ions were absorbed on the walls of the cage more rapidly than the positive. This fact accounts for the lower ion density in the negatively ionized atmospheres.

TABLE I. Succinoxidase Activity of Homogenates of Adrenal Glands from Rats Subjected to Various Stimuli. Six experiments.

Group	Succinoxidase (Qo_2, N*)	Deviation from control (%)	Significance of deviation, P values (11)
Controls (8)†	375 (±16.3)	—	—
Shock (4)	551 (±35.9)	+46.9	<.01
Positive ions (9)	325 (±39.9)	—13.3	<.01
Negative " (10)	389 (±42.8)	+ 3.7	.40
ACTH (5) (5 mg 4 hr previously)	415 (±36.7)	+10.6	.02
ACTH (6) (5 mg b.i.d. × 6)	307 (±37.0)	—18.1	<.01

* Mean values for μl oxygen uptake/mg tissue nitrogen/hr, including stand. error of each mean.
† Numbers in parentheses refer to number of animals in each group.

The series of experiments reported in Table I was conducted during an 8-week period in November and December. It will be noted that the succinoxidase activity of the control animals is significantly higher than had been found in the experiments on the effects of activity on adrenal succinoxidase reported above. These were performed during the summer months. It is possible that this is a

§ Qo_2, N; i.e., μl oxygen uptake/mg tissue nitrogen /hr. Figure in parentheses is standard error of mean calculated as S.E. $= \sqrt{\frac{\Sigma d^2}{n}}$.

reflection of seasonal changes in adrenal succinoxidase activity, since no other alteration in the experimental conditions or environment occurred during these 2 periods.

In the first experiments with positively or negatively ionized atmospheres, the succinoxidase activities were found to be very high. It was also noted that soon after installing the ionizer in the cage, the animal in the course of investigating this new addition to his surroundings invariably received an electric shock from the ionizing equipment. The polonium foil was then removed from the ionizer and the experiment repeated. Although no ionization was now present, the enzymatic activity 4 hours after installation of the ionizer without a polonium element was still significantly elevated (Table I). The results of this experiment served not only to modify the method for installation of the ionizer on the cage but also indicated the extent to which the succinoxidase activity of the adrenal would reflect the imposition of a stress stimulus. Following these experiments the ionizers were raised above the roof of the cages with a plastic cylinder, 5 inches in height. This device prevented direct contact of the body of the animal with the ionizer.

Animals were maintained in an atmosphere of positively or negatively ionized air for 4 hours. The effects of the ionized atmospheres on the adrenal succinoxidase activity are shown in Table I. In the case of those animals maintained in the positively ionized air, a statistically significant decline in succinoxidase activity was found, an effect opposite to that of the stimulus of an electric shock. Negatively ionized air produced a very slight rise which under the conditions of these experiments was not statistically significant. For comparison, the effect on adrenal succinoxidase measured 4 hours after the single subcutaneous injection of 5 mg of ACTH (Armour powder), or of 5 mg ACTH twice a day for 6 days was studied. The single ACTH injection produced a moderate rise in succinoxidase whereas after the repeated injections a significant fall was noted.

Summary. The effect of air ions on the succinoxidase activity of the rat adrenal gland has been studied. The activity status of the animal was found to affect the succinoxidase concentration of the adrenal. Electric shock produced a very significant rise. After 4 hours in positively ionized air the succinoxidase content of the rat adrenal was significantly reduced; a similar period in negatively ionized air produced a slight but insignificant rise. By comparison, a single injection of 5 mg of ACTH produced a moderate rise whereas the same dose given twice daily for 6 days resulted in a significant fall in adrenal succinoxidase.

1. Dessauer, F., *Ten Years Research in the Region Bordering on Physics and Medicine,* Georg Thieme, Leipzig, 1931.

2. Yaglou, C. P., Brandt, A. D., and Benjamin, L. C., *Heating, Piping, Air Conditioning,* 1933, v5, 423.

3. Skilling, H. H., and Beckett, J. C., *J. Franklin Inst.,* 1953, v256, 423.

4. Worden, J. L., *Fed. Proc.,* 1954, v13, 168.

5. ———, *Abst., Div. of Biol. Chem., Am. Chem. Soc.,* Spring Meeting, Kansas City, 1954.

6. Holloway, R. J., Thesis, University of San Francisco, 1952.

7. Rinfret, A. P., and Wexler, B. C., Report to Wesix Research Foundation, San Francisco, 1953.

8. McShan, W. H., Erway, W. F., and Meyer, R. K., *Arch. Biochem.,* 1946, v9, 69.

9. Schneider, W. C., and Potter, V. R., *J. Biol. Chem.,* 1943, v149, 217.

10. Hiller, A., Plazin, J., and Van Slyke, D. D, *ibid.,* 1948, v176, 1401.

11. Fisher, R. A., *Statistical Methods for Research Workers,* 10th ed., Oliver and Boyd, London, 1948.

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