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Absence of Eosinopenic Response to ACTH Administration by the Aerosol Route in Normal Subjects.* (19546)

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A number of investigators(1-5) have clearly demonstrated that the administration of ACTH in human subjects with unimpaired adrenal function produces a significant eosinopenia. This response has been employed as an index of adrenocortical function and in the assessment of the adrenocortical response to various forms of stress. In all instances, the hormone was administered either by the intramuscular or the intravenous route. The purpose of the present investigation was to study the effect of ACTH by the aerosol route upon the circulating eosinophils of normal individuals.

Plan of study. Six subjects (5 ♂ and one ♀), with ages ranging from 25 to 72, were selected. Two were free from any disease, one was affected with chronic bronchial asthma, but had been free from attacks for many months at the time of the experiment, and 3 had miscellaneous conditions (Table I). The entire group presented no signs of endocrine dysfunction, and all were permitted to carry on their usual routine during the period of investigation. An eosinophil count was taken on each of the subjects at 10 A.M., one hour after breakfast. This was considered the

initial or control count. In order to check the accuracy of the procedure, several of the counts were repeated by another observer whose results agreed. The Henneman modification(6) of the Randolph method was employed in the following manner: 2 cc of venous blood were drawn and placed in an oxalate-containing tube. A white-cell pipette was then filled to the 1.0 mark with oxalated blood. Phloxine-propylene diluent was next drawn up to the 11 mark, and the pipette mechanically shaken for about 2 minutes. Both sides of 2 Max Levy double counting chambers were filled, and the eosinophils in 4 ruled areas (comprising 16 sq mm each) were counted after the expiration of one-half hour, to allow for settling. The total of the 4 areas was then multiplied by a factor, 0.78. Following the control count, 25 mg of ACTH dissolved in 1 cc of distilled water were injected intramuscularly and an eosinophil count was taken 4 hours later. After an elapse of several days to insure complete dissipation of the drug, control eosinophil counts were again taken as described above, and immediately afterwards, 25 mg of ACTH dissolved in 1 cc of distilled water were given to each subject by the aerosol route. In 3 instances a De Vilbis No. 40 hand nebulizer was used, and in the remaining 3, a Vaponefrin nebulizer, at-

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TABLE I. Eosinophil Counts Before and 4 Hr After the Intramuscular Injection of 25 mg of ACTH (Aqueous).

Sub- ject	Age	Diagnosis	Initial count	Final count	% change
P.	25	No disease	197	44	-73
S.	28	Asthma (free from symptoms)	248	70	-72
R.	32	Latent lues	125	25	-80
G.	46	No disease	184	42	-77
C.	69	Arteriosclerotic H.D.	150	71	-53
F.	72	" , gen.	225	81	-64

TABLE II. Eosinophil Counts on G. Before and 2, 3 and 4 Hr After Aerosol Administration of 25 mg of ACTH (in Propylene Glycol).

Time of count	Eosinophil count	% change
Before ACTH	180	—
2 } hr after ACTH	200	+11
3 } hr after ACTH	200	+11
4 } hr after ACTH	220	+22

TABLE III. Eosinophil Counts Before and 4 Hr After Aerosol Administration of 25 mg of ACTH (Aqueous).

Sub- ject	Initial count	Final count	% change
P.	175	181	+ 3
S.	225	215	— 4
R.	138	131	— 6
G.	156	206	+32
C.	144	194	+34
F.	169	169	0

tached by a rubber tubing to an oxygen tank which employed 5 to 6 liters of oxygen per minute. On one subject (G.) additional eosinophil counts were performed 2, 3, and 4 hours after the aerosol administration of 25 mg of ACTH dissolved in propylene glycol (Table II).

Results. The control or initial eosinophil counts were in conformity with what is to be expected in normal individuals. All subjects demonstrated a normal decrease (50% or over) in circulating eosinophils ranging from -53% to -80% following the intramuscular injection of ACTH (Table I). However, following the administration of the hormone dissolved either in aqueous solution or in propylene glycol by aerosol route, no significant drop in the circulating eosinophils was observed (Table III). A slight decrease of 4% occurred in S. and one of 6% in R., whereas all other subjects demonstrated variable in-

creases. Such minimal alterations are frequently encountered in people irrespective of ACTH administration. A slight irritative cough was noted at the onset of the inhalation of the drug, and all subjects complained of a bitter taste. These symptoms were of short duration and in no way interfered with the completion of the test. The aqueous mixture was completely aerosolized in 5 minutes, whereas the procedure with ACTH dissolved in propylene glycol required 25 minutes.

Discussion. Hills, Forsham, and Finch(1) clearly demonstrated that a single intramuscular injection of 25 mg of ACTH causes a remarkably consistent eosinophil drop in normal subjects. They found that the decrease of the circulating eosinophils becomes apparent 2 hours after the injection of the drug, reaching its height at the end of 4 hours. They expressed this reduction as a percentage of the initial count and found it to be 50% or more in all instances of undisturbed adrenal cortical activity. Subsequently many other investigators confirmed this observation. Bonner and Homberger(7), employing the intravenous route of administration, obtained similar results with 5 mg of the hormone, and McIntosh and Holmes(8) with only 2.5 mg. The difference occurring with these 2 routes is explained by the fact that muscle tissue contains enzymes responsible for destroying the physiological activity of ACTH. The intramuscular route, therefore, does not appear to be the most efficient one for the administration of ACTH. The intravenous method, although requiring a much smaller dose, is cumbersome and is not entirely free from danger. An anaphylactic-like death has been reported from its use(7). The possibility of utilizing the aerosol route deserved investigation, therefore, since it would obviate these

difficulties, and, in addition, the hormone could be self-administered.

The administration of drugs by the aerosol route has long been an accepted mode of therapy. Many bronchodilators, antibiotics and the steroid hormone, cortisone, have been satisfactorily given by this method. The bronchopulmonary epithelium is highly vascular and offers an extensive area for absorption. When penicillin and other of the antibiotics are employed in this manner, blood levels comparable to those obtained when these agents are introduced either intramuscularly or intravenously are reached. There are some substances, however, which cannot penetrate this capillary barrier and are not absorbed by this means.

Rud(9), and Bonner and Homburger(7) demonstrated that simple fasting can produce a significant drop in the eosinophil levels in normal people. Hence, in order to overcome this physiological alteration, control counts were made shortly after breakfast.

The absence of adrenal insufficiency and the potency of the preparation used were indicated by previous response of the same sub-

jects to intramuscular administration of the same lot of ACTH. Thus, it is apparent that ACTH, given by the aerosol route, is not absorbed.

Summary. Observations on 6 normal subjects showed that eosinopenia did not occur following aerosol administration of ACTH, indicating that ACTH is not absorbed from the bronchopulmonary tree.

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Crystalline Material in Gastric Secretion. (19547)

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Following the report by Hollander(1) which described from light microscopy unidentified crystalline structures in dried smears of gastric juice, the attempt was made here to identify definitely the crystal molecule by means of electron and x-ray diffraction and to reproduce the dried, crystalline forms on electron microscope specimen screens. Human gastric secretion, obtained by Rehfuess tube from the stomachs of routine hospital patients was used in some experiments; specimens were also studied of mucus from Heidenhain pouch dogs obtained by topical stimulation with acetylcholine.*

Both the human and animal material were analyzed by electron and x-ray diffraction and

found to be at least 90% NaCl. Table I shows the x-ray data. With the exception of a few low intensity lines the NaCl values were reproduced exactly by the mucus. The few extra lines were not identifiable. The only identified compound in both x-ray and electron diffraction was NaCl.

Small drops of the mucus were placed upon prepared electron microscope screens and dried slowly in air, and in the resultant electron micrographs all of the forms observed in the light microscope were discovered. Fig. 1, 2, 3, and 4 illustrate the appearances. Rela-

* Samples of dog secretion were obtained from Dr. Franklin Hollander, Mount Sinai Hospital, N. Y. City.