at 270 m from the ocean. This led to an investigation of the dispersion of very tiny droplets into the atmosphere.

Since the cheesecloth traps did not give a measure of the relative size or number of droplets, a salt-sensitive paper was developed. Filter paper, of the 9-cm size, was dipped in 0.01 $N$ $K_2CrO_4$ and air-dried. The dried paper was then dipped in 0.02 $N$ $AgNO_3$, subsequently in distilled water to remove the excess $AgNO_3$, and redried. When droplets of sea water fell on the paper, light-yellow spots were formed by the chemical action between $Ag_2CrO_4$ and the halides of the sea water. The size of the spots is a relative measure of the size of the droplets, but is not a measure of the actual size of the droplets at the moment of impingement on the paper. For comparison of quantitative amounts of salt at each station, the paper was standardized by titration of samples with a known solution of NaCl. The difference between the titration value of the standardized samples and that of the exposed paper was taken as an indication of the quantity of salt caught at each station.

When the salt-sensitive paper was held above the oscillating swash between the breaking waves and the strand, it was almost immediately covered with small spots. These ranged from 4 mm in diameter to barely perceptible dots. When the paper was held above, or just in front of, a breaking wave the spots ranged from 4 to 20 mm in diameter and rarely showed evidence of small dots. It is then immediately apparent that the breaking waves do not disperse an appreciable number of tiny droplets into the atmosphere. It was thought, however, that the tiny droplets ejected into the air by the bursting bubbles of the swash and spume were small enough to be carried by the winds.

By using the oiled glass-slide method of Houghton and Radford (*10*), diameter measurements of air-borne droplets showed a range of 5–200 μ. The means of four determinations, totaling about 800 droplets, were between 35 μ and 55 μ. These droplets are well within the range of fog particles and are therefore easily transported by wind. When the frequencies of these droplets were plotted against diameters, Maxwellian-type curves similar to those of Stuhlman (*9*) were formed. This is considered to be further evidence that the majority of the air-borne droplets originated from bursting bubbles.

To obtain an indication of the area where the greatest quantity of salt became wind-borne, stations were located 5 m apart from the upper strand to beyond the breaking waves. Standardized salt-sensitive paper was thumbtacked to wooden stakes at a height of 50 cm above the strand and the water. Observations were made with a landward wind of 4–6 km/hr and with an outgoing tide.

The papers beyond the breaking waves showed negligible evidence of spray. One paper, when examined under 12-power magnification, showed several dots less than 0.5 mm in diameter. Above and immediately in front of the breaking waves, spots 4–20 mm were formed, with only an occasional dot less than 1 mm in diameter. Above the bursting bubbles of the swash numerous dots were formed, ranging up to 2 mm in diameter, with a mean of about 1 mm. Papers on the strand, 12 m from the highest edge of the water, showed the highest number of dots, but these were somewhat smaller than those of the swash.

Titration with NaCl did not show a significant difference in salt concentration between papers of the strand and those of the swash. This is possibly due to the larger number of smaller droplets caught on the strand. Those above the breaking waves showed the highest salt concentration because of large droplets being pitched by the breaking force of the waves. These droplets are considered to be too large to become air-borne and therefore do not contribute appreciably to atmospheric salts. The papers beyond the waves did not show a perceptible amount of salt. This does not mean that salts become air-borne only over the swash. It is evident that other disturbances on the open ocean which form small, bursting bubbles, such as foam produced by the wake of ships and whitecaps, would also be a source of atmospheric salts.

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## Eosinopenic Response of Adrenalectomized Mice to a Cutaneous Application of Cortisone


**Robert S. Speirs**[1]

*Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine*


Recent experiments have strongly indicated that the eosinopenia occurring over a 4-hr period is a specific response to adrenal cortical hormones. Certain strains of mice have been found to be extremely sensitive to these hormones and have been utilized in a procedure for assaying the 11-oxycorticosteroids (*1–4*). The eosinopenia is produced following subcutaneous, intramuscular, and intraperitoneal injections, as well as oral administration.

The reports of Baker and Whitaker (*5*) and Castor and Baker (*6*) indicated that cortisone produces a local action on the epidermis and connective tissue when applied cutaneously. It became of interest to ascertain whether this method of application also affected the eosinophils. The following report presents


[1] The technical assistance of L. E. Wragg, J. E. Sullivan, and B. Hadley is gratefully acknowledged.

TABLE 1

THE EFFECT OF CORTISONE ACETATE OINTMENT ON THE NUMBER OF CIRCULATING EOSINOPHIL CELLS OF PRETREATED-ADRENALECTOMIZED MICE*

| Treatment | Hr after cutaneous application of material | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | 1 | | 2 | | 3 | | 4 | | 6 | | 8 | | 10 | | 12 | | 20 | | 30 | | 50 | | 72 | | 96 | |
| | Eosinophil count | | Eosinophil count | Decrease (%) | Eosinophil count | Decrease (%) | Eosinophil count | Decrease (%) | Eosinophil count | Decrease (%) | Eosinophil count | Decrease (%) | Eosinophil count | Decrease (%) | Eosinophil count | Decrease (%) | Eosinophil count | Decrease (%) | Eosinophil count | Decrease (%) | Eosinophil count | Decrease (%) | Eosinophil count | Decrease (%) | Eosinophil count | Decrease (%) | Eosinophil count | Decrease (%) |
| Approximately 100 μg cortisone acetate in 4 mg ophthalmic ointment, applied to 9-cm² area of skin on dorsal region | 50 mice | | 112 | − 6 | 29 | −34 | 14 | −44 | 6 | − 84 | 2 | − 94 | 15 | − 51 | 22 | − 80 | 20 | − 37 | 129 | 0 | 196 | 0 | 276 | 0 | 205 | 0 | 128 | 0 |
| | | | 51 | 0 | 71 | −19 | 7 | −80 | 10 | − 74 | 8 | − 81 | 31 | − 40 | 17 | − 41 | 25 | − 42 | 187 | 0 | 234 | 0 | 171 | 0 | 124 | 0 | 240 | 0 |
| | | | 60 | −35 | 40 | −20 | 38 | −44 | 1 | − 99 | 4 | − 98 | 19 | − 44 | 35 | − 67 | 17 | − 82 | 125 | 0 | 266 | 0 | 182 | 0 | 232 | 0 | 143 | 0 |
| | | | 43 | −11 | 8 | −60 | 17 | −61 | 0 | −100 | 0 | −100 | 0 | −100 | 34 | − 75 | 25 | − 54 | 7 | −86 | | | | | | | |
| | | | 53 | 0 | 26 | −52 | 16 | −79 | 1 | − 96 | 5 | − 93 | 39 | − 51 | 3 | − 99 | 29 | − 49 | 34 | − 3 | | | | | | | |
| | | | 31 | − 9 | | | 6 | −88 | 4 | − 87 | 0 | −100 | 7 | − 83 | 23 | 0 | | | 8 | −79 | | | | | | | |
| Average | | 58 | 58 | −10 | 35 | −37 | 16 | −66 | 4 | − 90 | 3 | − 94 | 19 | − 62 | 22 | − 60 | 23 | −53 | 82 | −28 | 232 | 0 | 210 | 0 | 187 | 0 | 170 | 0 |
| Approximately 0.1 ml sesame oil applied to the skin on dorsal region | 44 mice | | 33 | 0 | 55 | 0 | 147 | 0 | 196 | 0 | 142 | 0 | 198 | 0 | 152 | 0 | 81 | 0 | 114 | 0 | 215 | 0 | 193 | 0 | 119 | 0 | 258 | 0 |
| | | | 82 | 0 | 69 | 0 | 75 | 0 | 182 | 0 | 295 | 0 | 103 | 0 | 105 | 0 | 68 | 0 | 146 | 0 | 223 | 0 | 240 | 0 | 271 | 0 | 317 | 0 |
| | | | 38 | 0 | 92 | − 2 | 229 | 0 | 221 | − 25 | 273 | 0 | 175 | 0 | 134 | 0 | 120 | − 25 | 146 | 0 | 202 | 0 | 214 | 0 | | | 173 | 0 |
| | | | 58 | 0 | 201 | 0 | 75 | 0 | 87 | 0 | 165 | 0 | 250 | 0 | 80 | 0 | 73 | 0 | 62 | 0 | | | | | | | | |
| | | | 42 | 0 | 150 | | 93 | 0 | 66 | 0 | 119 | 0 | 219 | 0 | 427 | 0 | | | 33 | −14 | | | | | | | | |
| | | | | | | | 124 | 0 | 64 | 0 | 179 | 0 | 227 | 0 | 58 | 0 | | | 160 | 0 | | | | | | | | |
| Average | | 53 | 51 | 0 | 113 | 0 | 123 | 0 | 136 | − 4 | 195 | 0 | 195 | 0 | 159 | 0 | 86 | − 6 | 103 | − 2 | 213 | 0 | 216 | 0 | 190 | 0 | 249 | 0 |

* The eosinophil counts presented here represent actual number of cells counted. To determine no. cells/mm³ of blood multiply by 5.

the results of single cutaneous applications of cortisone acetate on the eosinophils of adrenalectomized mice.

The animals used in these experiments were hybrid mice developed specifically for assaying adrenal cortical hormones. They were the $F_1$ offspring from a $C_{57}$ Brown/Jax and $C_{57}$ Black/Jax cross. Male mice weighing 20–25 g were adrenalectomized in a one-step operation and implanted subcutaneously with a 15-mg pellet of desoxycorticosterone acetate. Four or more days postoperatively the mice were pretreated with 20 μg of epinephrine (adrenalin chloride 1/5,000) and 4 hr later were massaged with either the cortisone ointment or sesame oil. (The ointment used was kindly supplied by F. Homburger.) It consisted of an ophthalmic base containing 25 mg cortisone acetate/g. Approximately 4 mg of ointment was applied on a shaved area of 9 cm² on the dorsal side of the mouse. Control mice were handled in the same manner but received 0.1 ml of sesame oil instead of the ointment.

Eosinophil counts were performed in a manner already described in detail (*1*). The number of these cells was determined in 94 mice just prior to the application of the cortisone ointment or sesame oil. The mice were then divided into groups of 3–6 animals, and different groups used for each subsequent count. In the majority of animals only 2 blood samples were taken, but a third sample was obtained from a few animals for the later counts (12–96 hr). Only animals having initial eosinophil counts of 60 or more cells/mm³ were used in this experiment.

The results obtained are tabulated and charted in Table 1 and Fig. 1. It can be seen that there was a marked eosinopenic response following the application of the cortisone ointment. This response began within 1 hr, reached a maximum at 4 and 6 hr and returned to normal after 20 hr. None of the controls showed any consistent decreases of eosinophils.

The data presented here indicate that the effect of the cortisone ointment applied to the skin is first measurable approximately 1 hr after administration and reaches a maximum at 4 hr. This is similar to the results obtained when cortisone is injected into the animals subcutaneously, intraperitoneally, or is given orally (*1, 7*). Additional experiments have indicated that as little as 3 μg of cortisone acetate in oil was effective in producing a marked eosinopenia when applied cutaneously. The cutaneous method of application was approximately as rapid and effective as the normally used subcutaneous injection. This method of application has possibilities for use in screening large numbers of oil-soluble organic compounds for adrenal cortical hormonelike activity. For example, some solvents, such as benzyl alcohol, are extremely toxic when injected subcutaneously or given orally, but have little or no toxic effect when applied cutaneously. Furthermore, only a single tellurite in concentrations that usually inhibit most the material to be screened—is necessary. With this modification it is possible to screen as many as 50 compounds per day with a working group of 4 trained technicians.

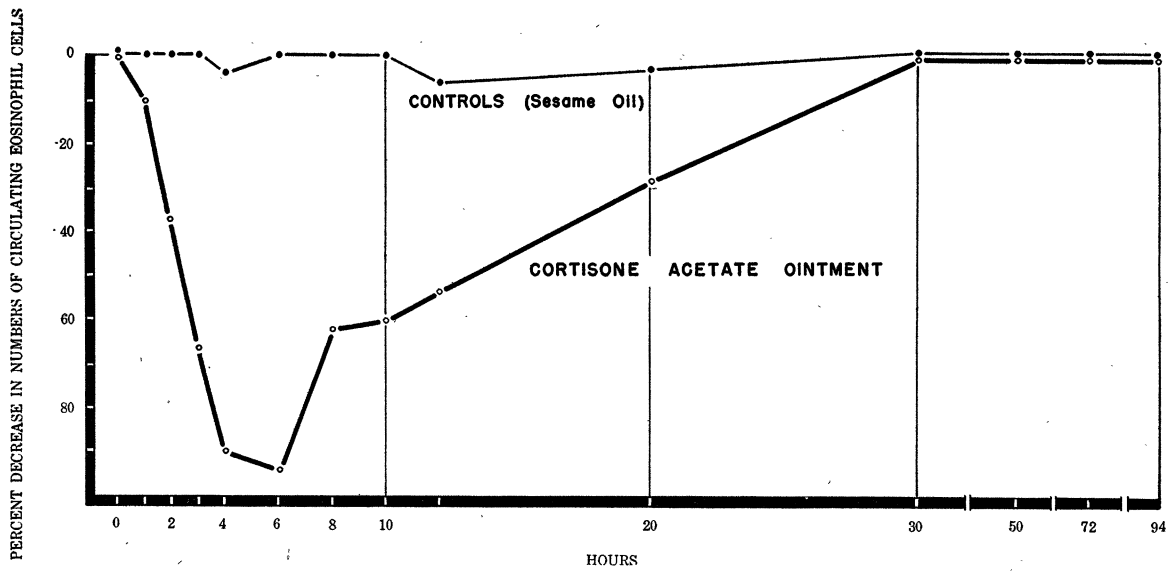


FIG. 1. Percentage decrease in number of circulating eosinophil cells following a cutaneous application of 100 μg of cortisone acetate.


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## Isolation of Pleuropneumonialike Organisms from the Throats of Humans


Paul F. Smith[1] and Harry E. Morton

*Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia*


Because of the frequent association of pleuropneumonialike organisms (PPLO), or L organisms, with a polyarthritis in rats and mice, earlier investigators quite logically looked for these microorganisms in humans exhibiting arthritic symptoms. In mice the conjunctiva, the mucosa of the nose, and occasionally of the trachea, and the brain serve as the habitat of the organisms (*1, 2*). When Sabin and Johnson (*2*) cultured the nose, throat, and conjunctiva of 100 individuals, they failed to find PPLO. In another attempt the above authors examined tonsils removed from 58 children for various reasons. A piece of tissue from each of the 116 specimens of tonsils was minced and then streaked over the surface of 30% ascitic fluid agar plates. In 3 of the 58 cases colonies 20–40 μ in diameter were observed among the bacterial colonies. These small colonies, designated *X* colonies, resembled those of PPLO. Impression smears of these small colonies were unsatisfactory and the *X* colonies never grew in subcultures. The nature of the *X* colonies remains unknown. It is possible that they were PPLO, as it has been observed recently that PPLO will grow in symbiosis with bacterial colonies (*3*).

At the time of their review in 1948, Dienes *et al.* (*4*) stated that PPLO had not been isolated from the human body from areas other than the genitourinary tract except when the individuals had received penicillin therapeutically or when penicillin was added to the culture medium to suppress the growth of bacteria. Dienes *et al.* stated that the PPLO in these cases represented a variant growth form of other bacteria produced under the influence of penicillin.

Smith *et al.* (*5*) discovered PPLO were not inhibited by crystal violet in concentrations that usually inhibit most gram-positive bacteria, nor by potassium tellurite in concentrations that usually inhibit most gram-negative bacteria. By adding both chemicals to an appropriate culture medium both gram-positive and gram-negative bacteria were inhibited, whereas PPLO grew readily. A suitable selective medium for PPLO was described by Morton, Smith, Williams, and Eickenberg (*6*) to consist of the infusion of 50 g Bacto-beef heart for infusion in 1,000 ml distilled water, to which are added 5 g NaCl and 10 g Bacto-peptone. After adjusting the pH to 7.8, 0.013 g crystal violet is added. Following sterilization in the autoclave at 121° C for 15 min, 0.53 ml Bacto-Chapman tellurite solution and ascitic fluid to a concentration of 25% of the final volume is added. This enrichment broth was employed with some of the throat cultures in these studies, but with a majority of the specimens a 1% concentration of a recently characterized serum fraction (*7*) was substituted for

[1] Difco Laboratories fellow in bacteriology.