

Thus the carcinogens, by inhibiting peroxide formation, may produce conditions favourable for growth.

MARY L. C. BERNHEIM
FREDERICK BERNHEIM
KARL M. WILBUR

Departments of Biochemistry,
Physiology and Pharmacology, and Zoology,
Duke University School of Medicine,
Durham, North Carolina.
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¹ Rusch, H. P., and Kline, E. J., *Cancer Res.*, **1**, 465 (1941).

² Bernheim, F., Bernheim, M. L. C., and Wilbur, K. M., *J. Biol. Chem.*, **174**, 257 (1948).

³ Wilbur, K. M., Bernheim, F., and Shapiro, O. W., *Arch. Biochem.*, **24**, 305 (1949).

⁴ U.S. Public Health Service Pub. 149 (1951).

⁵ Bernheim, F., Wilbur, K. M., and Kenaston, C. B., *Arch. Biochem. and Biophys.*, **38**, 177 (1952).

⁶ Stillwell, E. F. (unpublished results).

Precipitins to Grass Pollen Proteins

THE skin-reactive substances of grass pollen extracts used in clinical allergy have recently been shown to be proteins of average molecular weight 14,000¹. Only two instances of a precipitating antiserum to grass pollen extracts are known^{2,3}.

In the present investigation, the isolated protein components themselves were employed in experiments with rabbits and guinea pigs, aluminium phosphate⁴ and tubercle bacilli⁵ being used as adjuvants. Precipitin formation occurred in six out of seven rabbits and in all five of the guinea pigs injected.

Phleum pratense (timothy grass) and *Dactylis glomerata* (cocksfoot or orchard grass) pollen proteins were used as the immunizing antigens in doses varying from 50 to 400 mgm. per animal over periods from five weeks to several months. Injections were given subcutaneously or intramuscularly. At an antiserum dilution-level of 1/4, optimum flocculation points were within the antigen-range of 0.01-0.004 gm. pollen protein/100 ml., ranges of 0.1-0.000001 gm./100 ml. being tested. These are maximum figures, since none of the antigens used can be regarded as pure. The testing antigens were crude and purified pollen extracts of *Phleum pratense*, *Dactylis glomerata*, *Festuca pratensis* (fescue) and *Cynodon dactylon* (Bermuda grass). All the antisera and antigens were cross-reacting; but the amount of precipitate formed appeared to depend upon the degree of botanical relationship between the grasses.

Timothy pollen proteins have been fractionated into active albumins and globulins⁶. The globulins only were found to precipitate readily with the antisera to the unfractionated complexes. Accordingly, the supernatants from these antigen-antibody complexes were active throughout the testing range when used for skin (prick) tests on allergic subjects. At the optimum flocculation point, the activity in the supernatant was most probably due to uncombined pollen-albumin. The antigen-antibody complexes were washed vigorously with cold sterile saline. Even after repeating the process six times, positive skin reactions were obtained with the floccules dissolved in the minimum of 0.1 N sodium hydroxide.

It seems thus, that in timothy at any rate, precipitin formation is due to at least one of the skin-reactive proteins.

Work is in progress to put the precipitin reactions on a quantitative basis, with the view of standardizing

pollen extracts for clinical purposes in the treatment of asthma and hay fever.

ROSA AUGUSTIN*

Wright-Fleming Institute of Microbiology,
St. Mary's Hospital Medical School,
London, W.2. April 1.

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¹ Augustin, R., *Proc. Biochem. Soc.* (in the press; March 1953a).

² Augustin Friedmann, R., *Quart. Rev. Allergy and Applied Immunol.*, **6**, 290 (1952).

³ Marrack, J. R. (personal communication; 1952).

⁴ Holt, L. B., *Lancet*, **i**, 282 (1947).

⁵ Freund, J., and McDermott, K., *Proc. Soc. Exp. Biol. Med.*, **49**, 548 (1942).

⁶ Augustin, R., *Proc. Biochem. Soc.* (in the press; March 1953b).

A Source of Amniotic Fluid in the Lamb : the Naso-Pharyngeal and Buccal Cavities

INVESTIGATIONS in recent years have shown that there is an exchange of water between amniotic fluid and the blood streams of the mother and foetus. This has been observed in the guinea pig¹ and human². In the latter, a volume of water equal to about one-third the total volume of amniotic fluid is exchanged every hour from the twenty-eighth week to term³. At present, there is neither a set of observations nor a hypothesis to account for such a rapid and voluminous turnover of water, and no one has shown by what route or routes this fluid is derived.

During January to March 1953, extended observations in this laboratory were made on the reflex control of the circulation of the foetal lamb. A total of twenty-eight foetuses ranging in age from 110 days to term (149 days) were used. Most foetuses survived from one to four hours. During an experiment, the foetuses were delivered from ewes under 'Dial-urethane' anaesthesia by Caesarean section. Breathing of the lamb was prevented, with the placental circulation maintained, by applying a rubber nose-bag (condom) over the head of the foetus before it could breathe. This technique is like that used by Barcroft and Barron (see ref. 4), by Barclay, Franklin and Prichard⁵ and others. Before the nose-bag was applied, a small amount of amniotic fluid was placed in it.

In these experiments, we have been repeatedly impressed with the large volume of fluid accumulating in the nose-bag during the period of experimentation. The fluid is clear as water; but it contains a considerable amount of viscous material, quite clearly mucous in character. In the last two experiments, the volume of this accumulation was measured. The lambs were twins, weighing 2.3 kgm., estimated by weight to be about 120 days gestation age. In one experiment, 37 c.c. of fluid was added to an initial volume of 30 c.c. In the second, a volume of 29.5 c.c. was added.

The conditions of the first experiment were essentially as follows: the foetus was subjected to bilateral stellate ganglionectomy, with section of the third and fourth thoracic sympathetic post-ganglionic fibres, cannulation of the left carotid artery and bilateral adrenalectomy. A severe haemorrhage occurred during the last procedure. As a result, during the last part of a total period lasting 1½ hr. the foetus was moribund, with a blood pressure of 45/21 mm. of mercury. Despite this, the volume of fluid collected was 37 c.c.

The second foetus survived a total of 46 min. after application of the nose-bag. During this time the left