with anti-FRT specificity (Figure 4). The rise of 'non-anti-FRT' though small in comparison with that of 'non-anti-FRT' from other classes, was a constant feature of all but 4 immunized animals regardless of the route of administration of the antigen (Figure 4).

The data recorded here for IgG_1 and IgG_2 agree with previous reports of similar studies on conventional mice[7-8] and minimally contaminated mice[5] given ferritin by the parenteral route. In addition, specific antibody of the IgM and IgA types were found in some of the i.p. immunized animals. The data of SELL[9] and BAZIN[10] suggest that higher antibody titers might have been obtained in those mice receiving a single antigen dose, had longer intervals separated the bleeding from the immunizing injection.

The antibody response, in orally stimulated mice, being confined to the IgA class, further emphasizes the role of the IgA-producing lymphoid tissue of the gut in the immunological response to antigenic material present in the intestinal lumen.

This report also indicates that increases in immunoglobulins not reacting with the antigen frequently occur in all immunoglobulin classes, though they are proportionately most pronounced with IgM.

Résumé. Après injection de ferritine de cheval, des souris C_3H axéniques développèrent des anticorps circulants ainsi que des immunoglobulines sans affinité pour l'antigène dans chacune des classes: IgA, IgM, IgG_1, et IgG_2. Après immunisation orale, la réponse spécifique fut exclusivement du type IgA, et la réponse non-spécifique principalement du type IgG_2 et IgA.

D. R. NASH[11], P. A. CRABBÉ, H. BAZIN[12], H. EYSSEN[13] and J. F. HEREMANS

Department of Experimental Medicine, Cliniques Universitaires Saint-Pierre, Louvain (Leuven) (Belgium), 30 June 1969

[7] W. F. BARTH, C. L. MCLAUGHLIN and J. L. FAHEY, J. Immun. *95*, 781 (1965).
[8] F. L. ADLER, J. Immun. *95*, 39 (1965).
[9] S. SELL, J. Immun. *95*, 300 (1965).
[10] H. BAZIN, Ann. Inst. Pasteur *112*, 162 (1967).
[11] Belgian American Educational Foundation Fellow.
[12] Staff Member of the Biology Division of Euratom, Euratom Publication No. 486.
[13] The Rega Institute for Medical Research, Louvain.

Anti-Hapten Antibodies in the Serum and Bronchial Secretions of Dogs Following Respiratory Tract Immunization

During the past few years considerable information has accumulated concerning the secretory immunologic system. It has been demonstrated that IgA is the predominant immunoglobulin in the secretions of man[1-3], rabbits[4,5] and dogs[6].

It now appears possible to selectively stimulate the respiratory secretion immunologic system in man and to obtain protection against certain viral infections[7,8]. Only a single report exists showing the local induction of secretory IgA antibody directed toward a defined antigenic determinant. These investigators found that anti-dinitrophenyl (DNP) antibody of the secretory IgA class can be elicited by local injection of the antigen into rabbit mammary tissue[9].

The purpose of this investigation was to stimulate the production of secretory anti-hapten antibody and if possible to specifically purify and characterize the antibody.

Materials and methods. 3 adult mongrel dogs were used for this study. Immunization and the collection of blood and bronchial secretions were done while the dogs were under sodium pentothal anesthesia. To obtain bronchial secretions, 10 ml of phosphate buffered saline was instilled via a bronchoscope and immediately removed by suction. Approximately 6 ml was recovered from each dog. The 3 washes were pooled, homogenized in a tissue grinder, centrifuged and concentrated to 3 ml. Serum samples were also pooled (equal volumes from each dog).

During a 12-week period the dogs received 3 intratracheal injections of 2.5 mg 2,4-dinitrophenylated keyhole limpit hemocyanin (DNP-KLH). 4 weeks later another intratracheal injection was given and DNP-KLH was administered by aerosol. Samples of blood and bronchial secretions were obtained 3 weeks later and an additional 10 mg DNP-KLH was given via a bronchoscope. Blood and bronchial washes were obtained 5 weeks following the final antigenic challenge.

Sera and bronchial washes were assayed for anti-DNP activity using a modified phage neutralization assay, employing DNP-T_4 bacteriophage conjugates[10]. The specificity of the neutralization reaction was determined by: (a) preincubating dilutions of selected samples with $2\times10^{-2}M$ ε-DNP-caproic acid for 1 h at 37°C and comparing the neutralizing capacity with serum and bronchial aliquots diluted with saline in place of the hapten inhibitor; (b) testing samples against unconjugated T_4 bacteriophage.

One immune serum pool was assayed in order to establish the kinetics of DNP-T_4 neutralization. The Figure shows that under the conditions of these experiments neutralization followed first-order kinetics for at least 10 min. Thus all samples were assayed using 10 min neutralization times. The potency of the various samples is expressed in terms of the rate constant for phage inactivation:

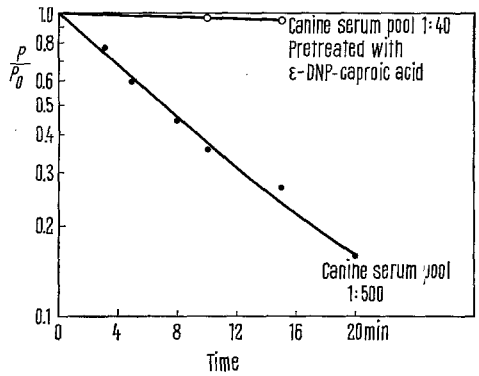
$$K = \frac{-1n\,(p/p_0)}{C\Delta T}$$

where p = number of plaques at time t; p_0 = number of

[1] W. B. CHODIRKER and T. B. TOMASI, Science *142*, 1080 (1963).
[2] J. S. REMINGTON, K. L. VOSTI, A. LIETZE and A. L. ZIMMERMAN, J. clin. Invest. *43*, 1613 (1964).
[3] T. B. TOMASI, E. M. TAN, A. SOLOMON and R. A. PRENDERGAST, J. exp. Med. *121*, 101 (1965).
[4] J. J. CEBRA and J. B. ROBBINS, J. Immun. *97*, 12 (1966).
[5] J. J. CEBRA and P. A. SMALL, Biochemistry *6*, 503 (1967).
[6] J. S. JOHNSON and J. H. VAUGHN, J. Immun. *98*, 923 (1967).
[7] R. H. WALDMAN, J. J. MANN and P. A. SMALL, J. Am. med. Ass. *207*, 520 (1969).
[8] L. P. TREMONTI, L. L. JUEY-SHIN and G. G. JACKSON, J. Immun. *101*, 572 (1968).
[9] R. J. GENCO and M. A. TAUBMAN, Nature *221*, 679 (1969).
[10] The DNP-T_4 conjugate used was kindly provided by Dr. O. MÄKELÄ. O. MÄKELÄ, Immunology *10*, 81 (1966).

plaques at time $t = t_0$; c = sample dilution factor; and ΔT = time in min $(t - t_0)$.

Results. Following 4 intratracheal injections and one administration of antigen by aerosol, the serum pool and the bronchial secretion pool had a K of 50.5 and 0.37 respectively, with T_4-DNP. These samples each gave a K of approximately 0.02 with unconjugated T_4. Following the final bronchial administration of antigen, serum had a K of 3.9 and bronchial wash gave a $K = 46$. As before, K's of approximately 0.02 were obtained with unconjugated T_4. The neutralizing activity of the anti-DNP samples could be almost completely inhibited by preincubating them with ε-DNP-caproic acid (Figure).



Kinetics of DNP-T_4 neutralization with canine anti-DNP serum. P/p_0, ratio of plaques remaining at time t to original number of plaques. The number following the sample name refers to the dilution of antiserum used.

Discussion. Intratracheal and aerosol immunization of dogs with a DNP-protein conjugate resulted in circulating anti-DNP and low levels of anti-DNP in the bronchial secretions. Following the final direct instillation of DNP-KLH into the respiratory tract a definite increase in anti-DNP activity in the secretions was noted whereas the circulating anti-DNP level dropped. This may have been due to the selective stimulation of antibody producing cells in the respiratory tract. WALDMAN et al.[11] have shown that the antibody response varies with particle size; the smaller particle sizes give better serum antibody responses, that larger particles give better levels of secretory antibody.

Previous authors working with dogs have demonstrated that there is a preferential secretion of IgA in canine milk, saliva and bronchial mucus[6,12]. Our object was to specifically purify the 'secretory' anti-DNP antibodies and to characterize them as to immunoglobulin class, valence and association constant. However, the extremely low levels of specific anti-DNP made this unfeasible. This study points out that anti-DNP antibodies can be detected in the secretions of dogs. Careful studies on the particle size, route of administration, and dose of antigen employed will be necessary in order to establish a method which will yield high levels of secretory anti-hapten antibody. Detailed physical chemical studies can then be carried out.

Zusammenfassung. Im Serum und in Bronchialsekreten von Hunden konnten Antikörper gegen DNP, die über die Atmungswege mit DNP-Proteinkonjugaten immunisiert worden waren, nachgewiesen werden. Die Titer der beobachteten spezifischen Antikörper waren für eine detaillierte Analyse zu niedrig.

G. A. LESLIE[13] and R. H. WALDMAN

Departments of Microbiology and Medicine, College of Medicine, University of Florida, Gainesville (Florida 32601, USA), 6 June 1969

[11] R. H. WALDMAN, R. CARROLL, E. TORRES and S. H. WOOD, Am. Rev. resp. Dis., submitted for publication.

[12] J. P. VAERMAN and J. F. HEREMANS, Immunochemistry *5*, 425 (1968).

[13] This work was supported by National Institutes of Health Training Grant No. 5TI AI0128-09.

A Note on the Neurosecretory Pathways in *Pyrilla perpusilla* Walker (Fulgoridae: Homoptera)

GABE[1] in his book 'Neurosecretion' mentioned: 'the microscopic anatomy of the stomatogastric system and the retrocerebral endocrine glands in Homoptera would appear to have less study than the anatomy of the same organs in Heteroptera'. This prompted me to undertake a study of the neuroendocrine system of the sugarcane leaf hopper, *Pyrilla perpusilla* Walker, the details of which will be presented elsewhere. During the course of these investigations stained material was invariably seen in the intercellular spaces of the neuropillar tissue of the brain. This material had the same staining properties as the neurosecretory material (NSM) revealed in the neurosecretory cells of the protocerebrum. In this paper the distribution and significance of the intercellular material will be described briefly.

Adults of *P. perpusilla* were collected from sugarcane fields. The required organs were fixed and stained with aldehyde-fuchsin (AF) or performic acid-victoria blue (PAVB) in the manner described by DOGRA and TANDAN[2]. The preparations were supplemented by histological sections stained either before (PAVB) or routinely after sectioning (AF).

Two groups of A-type cells were found in the brain. These cells stained deep purple and greenish-blue with the AF and PAVB techniques respectively. The groups are located on the ventral side of the pars intercerebralis medialis; this in contrast to their location in other insects where they are usually found in an antero-dorsal position. Each group consists of 12–16 cells. When the A-cells were loaded with NSM, their neurosecretory pathways were visible (Figure 1). In such preparations stained colloids or granules were seen on the postero-lateral margins of the A-cells (Figures 2 and 3), but were never observed in brain regions anterior to these cells. The staining intensity of the material visible in the intercellular spaces of the brain varied in preparations from different insects, but was directly proportional to the amounts of NSM in the neurosecretory cells of the same individuals (Figures 2

[1] M. GABE, 'Neurosecretion', Pergamon Press, London, New York (1966).

[2] G. S. DOGRA and B. K. TANDAN, Quart. J. micr. Sci. *105*, 455 (1966).