(1:1.3), as previously suggested by FOLKES et al.[11], meanwhile in the polysaccharidic fraction from *B. vulgaris* there is a much larger amount of galactosamine (glucosamine-galactosamine ratio 1:8.8) (Figures 1 and 2).

It seems to us that in the egg gelatinous mantle of these 2 species sialofucopolysaccharides are present with variable amounts of hexosamines (glucosamine and galactosamine), indicating a different specific composition of the sialopolysaccharides.

Riassunto. Sono stati studiati con analisi biochimiche gli involucri ovulari di *R. latastei* e *B. vulgaris*, soprattutto in relazione alla presenza di esosamine e acidi sialici. Sono presenti sia glucosamina che galattosamina con un rapporto in *R. latastei* di 1:1,3 e in *B. vulgaris* di 1:8,8. Gli acidi sialici sono contenuti in quantità elevata così pure il glucosio, mentre sono assenti acido glucuronico e SO_4^{--}. Si è potuto quindi concludere che negli involucri ovulari delle due specie considerate sono presenti sialofucopolisaccaridi con quantità variabili di esosamine.

L. BOLOGNANI, A. M. BOLOGNANI FANTIN, R. LUSIGNANI, and L. ZONTA

Istituto di Chimica Biologica dell'Università di Pavia, Istituto di Anatomia Comparata dell'Università di Pavia e Centro di Studio per l'Istochimica del CNR, Pavia (Italy), December 3, 1965.

Transfer of the Immunization to a Bacterial Antigen by RNA

By experiments on the transfer of nucleic acids between different cellular populations, data have been obtained on the acquisition of new immunological properties by normal lymphoid cells after treatment with RNA or subcellular fractions extracted from immunized animals[1-5]. It has also been proved that spleen cells from normal mice acquire the ability to synthesize sheep hemolysin after incubation in vitro with RNA extracted from the spleens of isologous donor mice immunized by sheep erythrocytes[6-8]. The acquisition of the immunological capabilities is strictly specific. Thus the ability to synthesize sheep hemolysin is absent in spleen cells from normal mice incubated with RNA obtained from donors immunized with chicken erythrocytes or bovine serum-albumin[8]. The relation between the extracellular-RNA and antibody-globulin synthesis is proved also by the demonstration of an evident increase of the content of RNA in the γ-globulin fraction in immune sera[9].

Immunization transfer in vivo by means of RNA has also been proved feasible by a research programme performed in our laboratory. As a matter of fact, when RNA obtained from the spleens of animals that had been immunized with ram erythrocytes was injected into normal animals, antibodies that could agglutinate ram erythrocytes were found in the latter's serum[10]. Analogous results have been obtained in different experimental conditions. In fact it has been demonstrated that the RNA extracted from the serum of immunized animals is capable of eliciting in normal recipient animals the production of antibodies against the same antigens used for immunizing the animals from which this RNA-immuno-carrier was taken[11].

The research work related in this paper was aimed at assessing the possibility of using RNA for transferring immunization against a bacterial antigen from one animal to another. In this class of investigations, the most serious difficulty arises from the limited amount of RNA that can be obtained from the spleens of immunized animals, particularly when only the nucleolar RNA 2nd fraction is used as the portion endowed with the highest activity. On account of this, our experiments were performed by extracting RNA from the spleens of a large number of immunized adult subjects and injecting it into new-born animals so as to obtain the highest possible RNA concentration in the plasma of the recipient subjects. Animals originating from the same stock were used throughout the experiment.

Methods of immunization and RNA extraction. 50 rabbits averaging 3 kg in weight were administered 5 i.v. injections (1 every third day), each consisting of 5 ml of a suspension of *S. typhi* 'H' antigen. The antigen had been prepared from agar cultures of *S. typhi* H 901 treated with formalin, the opacity being equivalent to that of the first tube of Wellcome's opacity meter. 20 days after the beginning of the immunizing treatment, antibody titrations were in excess of 1/70,000 in all subjects. At that time the rabbits were killed and RNA was immediately extracted from their spleens by the GEORGIEV and MANTIEVA method[12,13].

Characteristics of the RNA employed. In the experiment only nucleolar RNA 2nd fraction was used. The chemical composition of this fraction was as follows: RNA 96.8%; proteins 1.5%; polysaccharides 0.8%; DNA 0.9%. For the control of the molecular integrity of RNA it was characterized by the hyperchromic effect: Optical density (OD) of the native RNA 2.07 (260/280 nm); 2.26 (260/230 nm); OD after alkaline hydrolysis 1.81 (260/280 nm); 2.41 (260/230 nm).

Treatment of the recipient rabbits. Some of the RNA so obtained was dissolved in a salt-free 5% dextrose solution and injected i.v. (dosage: 10 mg/kg body weight) into 5 new-born rabbits. The injections were repeated

[1] M. FISHMAN, J. exp. Med. *114*, 837 (1961).
[2] J. A. MANNICK, Ann. Surg. *156*, 356 (1962).
[3] J. A. MANNICK and R. M. EGDAHL, Science *137*, 976 (1962).
[4] J. FONG, D. CHIN, and S. S. ELBERG, J. exp. Med. *118*, 371 (1963).
[5] H. FRIEDMAN, Biochem. biophys. Res. Commun. *17*, 272 (1964).
[6] S. ESPOSITO, L. BUSCARINI, E. NICOLINI, E. L. CHÉRIÈ LIGNIÈRE, and P. MARANDOLA, Riv. Emoterap. Immunoemat. *11*, 183 (1964).
[7] E. P. COHEN and J. J. PARKS, Science *144*, 1012 (1964).
[8] H. FRIEDMAN, Science *146*, 934 (1964).
[9] L. MICHELAZZI, G. NANNI, I. BALDINI, and A. NOVELLI, Experientia *20*, 447 (1964).
[10] S. ESPOSITO, E. L. CHÉRIÈ LIGNIÈRE, and E. POZZI, Lancet *II*, 444 (1965).
[11] L. MICHELAZZI, I. BALDINI, A. NOVELLI, and G. NANNI, Nature *205*, 194 (1965).
[12] G. P. GEORGIEV and V. L. MANTIEVA, Biokhimiya *25*, 143 (1960).
[13] G. P. GEORGIEV and V. L. MANTIEVA, Biochim. biophys. Acta *61*, 153 (1962).

three times at 3-day intervals, and antibody titrations in the subjects' serum were determined 2 days after the last RNA injection.

Controls. In order to detect the possible presence of antigen or of antibodies in preparations, the RNA was separated from admixtures. For this purpose the RNA was treated with warmed ribonuclease (10 μg/100 μg RNA) and then dialysed against a 0.15 M solution of NaCl for 5–6 h at 37 °C and then for 10–12 h at 4 °C for the complete removal of the degraded RNA. The residue remaining after dialysis was injected intravenously (dosage: 2 mg/kg body weight) into 5 new-born rabbits. The injections were repeated 3 times at 3-day intervals. Antibody titrations were determined 2 days after the last injection. Finally, antibody titrations were determined on the serum of 20 new-born rabbits that had received no RNA treatment.

Results. After injections of RNA obtained from the spleens of animals that had been immunized with *S. typhi* 'H' antigen, antibody titrations in the serum of new-born recipient subjects were found to be 1/30,000, 1/35,000, 1/25,000, 1/30,000 and 1/40,000. No antibodies were found, however, in the serum of the new-born control subjects or of the new-born animals injected with the residue obtained after destruction and removal of RNA. Therefore only the RNA molecule in its native state is the agent of the immunization transfer, while the small percentages of proteins, polysaccharides and DNA injected together with RNA had no influence. The last result is in accordance with the data obtained by FUKS et al.[14].

For the time being, the difficulty of obtaining an adequate supply of RNA from the spleens of immunized animals does not allow an all-round picture to be obtained of how the phenomenon occurs – when antibody production begins, how long it lasts, which are the minimum active dosages, etc. Even though the experiment has so far been performed with inadequate supplies of experimental material the results nevertheless seem clearly to suggest that the possibility does exist of obtaining an antibody synthesis in vivo by means of RNA.

Riassunto. L'iniezione ad animali normali di ARN estratto dalla milza di animali immunizzati con antigene 'H' di *S. typhi*, provoca la comparsa, nel siero degli animali riceventi, di anticorpi diretti contro tale antigene.

S. ESPOSITO and E. L. CHÉRIÈ LIGNIÈRE

Clinica Medica dell'Università di Pavia (Italy), September 30, 1965.

[14] B. B. FUKS, I. V. KONSTANTINOVA, and A. P. TSYGANKOV, Vest. Akad. med. Nauk SSSR *19*, 28 (1964).

The Tonically Acting Pulmonary Receptors Innervated by C Fibres

The investigation of the activity of single afferent vagal fibres has revealed pulmonary stretch receptors (ADRIAN[1]) which are innervated by A fibres. However, the majority of vagal afferent fibres, about 80%, including afferent fibres from the lungs, belong to C fibres (AGOSTONI et al.[2]). The function of these fibres up to now remained obscure.

In 1955 and 1957 PAINTAL[3,4] established the existence of vagal pulmonary afferent fibres excited by deflation of the lung and supposed that these fibres innervated pulmonary deflation receptors. By their conduction velocity these fibres belonged to B fibres. But in 1964 the author reported that most of them belonged to C fibres.

In 1965 COLERIDGE et al.[5] distinguished vagal pulmonary afferent fibres which also belonged to C fibres. It is very important to emphasize that according to the authors' findings these fibres show spontaneous activity which is not synchronized with the cycles of breathing. COLERIDGE et al.[5], contrary to the data obtained by PAINTAL[3,4], found that the deflation of the lungs did not excite these fibres but that they were excited by hyperinflation.

The observations of the influence exerted by some drugs evoking respiratory chemoreflexes on these fibres are also contradictory. According to PAINTAL[6], phenyl diguanide excited these fibres while veratrine did not. Quite the contrary is observed by COLERIDGE et al.[5]. In their experiments veratrine excited C fibres while phenyl diguanide did not.

The preparation of single C nerve fibres is very difficult because they are very thin and are often injured. Therefore we used the method of colliding impulses (DOUGLAS and RITCHIE[7]). This method has also another advantage: it permits us to establish the function of the greatest part of fibres of a definite group.

Method. The experiments were made on 25 cats weighing 2.0–4.0 kg anaesthetized with urethane and chloralose. The chest was widely opened and artificial respiration was carried out. The right cervical vagus was cut just below the nodose ganglion. A pair of stimulating platinum electrodes was placed close to the peripheral cut end of the nerve, and another pair of recording electrodes was placed about 50–90 mm lower. For stimulation of the nerve rectangular pulses of current of 0.1–0.5 msec duration were used, the stimuli used being supramaximal for A or C components. The vagus was stimulated during the inspiration or expiration. To exclude the impulses from other organs, the left and right vagi were cut over the diaphragm and the majority of cardiac branches of the right vagus were cut too. Thus only the excitation of pulmonary receptors may act on the compound action potential of vagus.

[1] E. D. ADRIAN, J. Physiol. *79*, 332 (1933).
[2] E. AGOSTONI, J. E. CHINNOCK, M. DALY DE BURGH, and J. G. MURRAY, J. Physiol. *135*, 182 (1957).
[3] A. S. PAINTAL, Q. Jl. exp. Physiol. *40*, 2 89 (1955).
[4] A. S. PAINTAL, Q. Jl. exp. Physiol. *42*, 1, 56 (1957).
[5] H. M. COLERIDGE, J. C. G. COLERIDGE, and J. C. LUCK, J. Physiol. *179*, 248 (1965).
[6] A. S. PAINTAL, Pharm. Rev. *16*, 4, 341 (1964).
[7] W. W. DOUGLAS and J. M. RITCHIE, J. Physiol. *138*, 19 (1957).