variation in time and its dependence upon different schemes of immunization. The cell mediated immunity appears first; therefore there does exist a brief period when the reaction can be considered unidirectional; later, the humoral response may predominate. This finding should be taken into account in attempts to perform tumour immunotherapy in man[17].

[17] This investigation was supported by grants from the Consiglio Nazionale delle Ricerche, Rome. We thank Mr. L. FRANCHI for excellent technical assistance and Miss LUISA BENNI for useful advice.

Résumé. Une modification de l'immunogénicité des TSTA a été obtenue par l'hybridation des cellules d'un sarcome de rat (souche Fisher) avec des cellules normales allogéniques. La réponse des animaux syngéniques (immunisés avec les hétérokaryons) à l'implant de la tumeur fut strictement dépendante du schème d'immunisation.

G. BARBANTI-BRODANO, A.T. DI MARCO, L. POSSATI, C. FRANCESCHI and G. PRODI

Institute of Microbiology and Institute of Oncology, University of Bologna, Via San Giacomo 12, I–40126 Bologna (Italy), 15 May 1974.

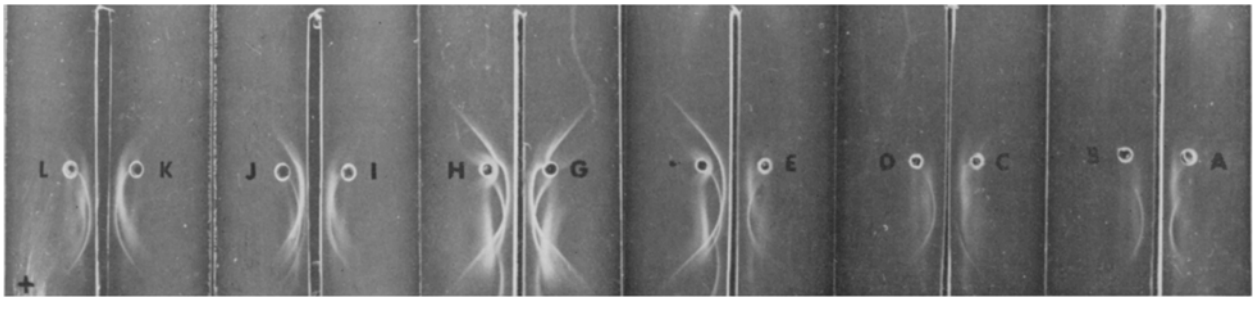
Alteration of Antigenic Components of Rat Parotid Gland Following Denervation

Removal of the innervation to salivary glands is followed by changes in the denervated and the normally innervated contralateral mate[1]. The characteristics of these changes depend however on the kind of denervation effected. Thus, the structural and functional modifications following removal of the superior cervical ganglion are not marked, while those following parasympathectomy are[2]. However, there are growth responses in the sympathectomized gland that are not found in the parasympathectomized gland[3]. These growth responses are attributed in part to circulating entities described nonspecifically as humoral factors[4,5]. It has recently been shown that removal of the superior cervical ganglion results in alteration of the immunoelectrophoretic pattern of the serum characterized by the appearance of additional antigenic bands[6]. The present work has been undertaken to determine whether these alterations were a reflection of antigenic changes in the gland itself.

Methods. Female Long-Evans rats, 6 months old, maintained on lab chow and water ad libitum were used in these experiments. Under light anesthesia, one of the following surgical procedures was performed: unilateral removal of the superior cervical ganglion (Sx); removal of a portion of the auriculotemporal nerve (Px); or a combination of these two procedures (PxSx); controls were either unoperated rats or those on which sham operations were done. In addition a group of unoperated rats was placed on a dietary regimen of liquid diet, Metrecal, (ME). 2 weeks after surgery or maintenance on liquid diet the paired parotid glands from animals of all groups were removed under Nembutal anesthesis (50 mg/kg, i.p.). The glands of each group were homogenized in saline, and the homogenate was then centrifuged at 10,000 rpm for 10 min and the supernatant was collected. Total protein content of the extracts was determined by the method of LOWRY et al.[7] and protein content of different extracts was adjusted to a concentration of 20 mg/ml.

Antisera were prepared by injecting saline extract of normal rat parotid gland s.c. into rabbits. The extract was incorporated into Freund's complete adjuvant for the first injection and into incomplete adjuvant for subsequent injections, as previously described[8]. Antiserum was absorbed with lyophilized rat serum in a concentration of 70 mg/ml of rabbit serum, which was found to be a proper concentration for neutralization by preliminary gel diffusion tests. Two-dimensional immunodiffusion was performed according to the method of OUCHTERLONY[9]. Immunoelectrophoresis was carried out according to the method of GRABAR and BURTIN[10] as modified by SCHEIDEGGER[11], using 1% 0.05 *M* barbital buffer at pH 8.6.

[1] C. A. SCHNEYER, in *Regulation of Organ and Tissue Growth* (Ed. R. Goss; New York, Academic Press 1972), p. 211.
[2] A. S. V. BURGEN and N. G. EMMELIN, in *Physiology of the Salivary Glands*, (Arnold, London 1961).
[3] C. A. SCHNEYER, Proc. Soc. exp. Biol. Med. *142*, 542 (1973).
[4] A. ALHO, Acta path. microbiol. scand., Suppl. *149*, 11 (1961).
[5] A. LACASSAGNE and R. CAUSSE, C.r. Soc. Biol., Paris *135*, 241 (1941).
[6] C. A. SCHNEYER, S. EL-MOFTY and W. J. BYRD, in press.
[7] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[8] S. EL-MOFTY, Ala. J. med. Sci. *9*, 437 (1972).
[9] O. OUCHTERLONY, Microbiologia scand. *25*, 186 (1948).
[10] P. GRABAR and P. BURTIN, *Analyse Immuno-Electrophoretique* (Masson and Cie, Paris 1960).
[11] J. J. SCHEIDEGGER, Int. Arch. Allergy *7*, 103 (1955).



Immunoelectrophoresis in agar gel. Troughs contain rabbit anti-parotid gland extract absorbed with rat serum. Rat parotid gland extracts in wells (protein concentration 20 mg/ml): A) normal; B) parasympathectomized (Px); C) contralateral to parasympathectomized; D) parasympathectomized (Px); E) normal; F) sympathectomized (Sx); G) contralateral to sympathectomized; H) sympathectomized (Sx); (I) totally denervated (PxSx); J) contralateral to totally denervated; K) totally denervated (SxPx); L) maintained on liquid diet (ME).

Results and discussion. Immunodiffusion and immunoelectrophoretic studies on saline extracts of parotid glands removed 2 weeks after selective denervation or maintenance of rats on liquid diet showed that alterations in the antigenic profile of the gland were caused by each of these procedures. The most marked changes were effected by denervation involving either unilateral removal of a superior cervical ganglion, or that involving removal of part of the auriculotemporal nerve. A striking increase in concentration of the various saline soluble antigens was found in sympathectomized glands, whereas parasympathectomy resulted in a deletion of the majority of the antigenic components (Figure). With denervation involving both of these surgical procedures (PxSx), or with maintenance of animals on liquid diet, only slight alterations from the normal profile were observed. With the exception of Px, normally innervated mates to the denervated glands also showed marked changes. Thus, the innervated mate to Sx glands showed a marked increase in the antigenic components that was very similar to the changes seen with the Sx gland itself. Increases in the concentration of antigenic components were also seen in the control mates to PxSx glands.

The antigenic alteration noted in the Px glands is not unexpected, since the deletion of antigens is paralleled by glandular atrophy[3]. On the other hand, sympathectomy also ultimately results in mild atrophy[12]. However, the changes in antigenic profile do not reflect deletions but rather increase in concentration of the antigenic components. This difference may be related to the early growth changes manifested by the Sx glands. Marked mitotic activity occurs within 2 days after sympathectomy and by 4 days, cell number is greatly increased[3]. As a result of these changes, an increase in concentration of antigenic components could be expected. In fact, the altered serum pattern found following sympathectomy may also be a reflection of the altered antigenicity of the Sx gland[6]. Furthermore, it is interesting that this alteration of serum pattern characterized by the appearance of additional antigenic bands is associated with augmentation of the antigenic components present in the parotid gland. These findings raise the possibility that these additional serum antigens originate from the gland.

The antigenic components of an organ have been suggested to play a role in regulating the growth response of this organ. The present findings suggest that an association between the antigenic characteristics of the gland and its growth potential exists[13]. Further investigations aimed at clarification of such a relationship are being carried out.

Résumé. Altération des caractères de la glande parotide du rat par dénervation séléctive ou après diète liquide. A la suite de l'ablation unilatérale du ganglion cervical supérieur, une augmentation marquante de la cocentration des composants antigéniques solubles dans le salin se produisit aussi bien dans la glande parotide denervée que dans la glande intacte. Par ailleurs, après l'ablation partielle du nerf auriculotemporal, les composants antigéniques de la glande parotide denervée diminuèrent. La corrélation possible entre ces changements et le développement de la glande parotide est discutée.

S. El-Mofty and Charlotte A. Schneyer[14]

Department of Physiology and Biophysics, University of Alabama in Birmingham, Birmingham (Alabama 35294, USA),
30 January 1974.

[12] C. A. Schneyer and H. D. Hall, in *Secretory Mechanisms of Salivary Glands* (Eds. L. H. Schneyer and C. A. Schneyer; Academic Press, New York 1967), p. 155.
[13] M. M. Burger, in *Current Topics in Cellular Regulation* (Eds. B. L. Horecker and E. R. Stadtman; Academic Press, New York 1971), vol. 3.
[14] Acknowledgment. This work was supported in part by a USPHS grant No. DE 02110.

Actinomycin Binding and Uridine Incorporation by Human Normal Bone Marrow Cells

Due to its continuous renewal, normal bone marrow offers a very favorable model for the investigation of differentiation in Eukaryotic cells. Its study is also a necessary prerequisite for the comparative analysis of normal cells and their leukaemic counterparts. Unfortunately, biochemical approaches are made exceedingly difficult, in this case, by the high heterogeneity and variability of the marrow population; therefore, autoradiography and cytochemistry still remain the main lines of approach. Auroradiography has already shown that differentiation of the myeloid and erythroid lines is accompanied by the loss of DNA replication and a gradual tailing-off of RNA and protein synthesis[1-3].

An evaluation of the number of DNA molecule sites free for RNA-polimerase can now be made by measuring ^{3}H-actinomycin D binding in single nuclei. This technique suggests that there is a close correspondence between actinomycin binding and RNA synthesis, particularly messenger RNA and transfer RNA synthesis[4]. Increased chromatin 'template' activity, resulting in enhanced RNA production, is, as a rule, accompanied by increased actinomycin D binding[5-6]; restriction of genetic activity occurring in the advanced stages of differentiation, on the contrary, is accompanied by reduced binding[7].

Further work in this direction is now reported in the form of an autoradiographic comparison of tritiated actinomycin D binding and uridine-5-T incorporation into RNA of normal myeloid and erythroid cells.

Material and methods. Uridine uptake and ^{3}H-actinomycin binding were evaluated on bone marrow cells from 2 normal subjects. The in situ technique of Brachet and Ficq was used for actinomycin binding[8-9]. Cell smears were immediately fixed in 95% ethyl alcohol and acetic

[1] F. Gavosto, A. Pileri and G. Maraini, Haematologica *44*, 976 (1959).
[2] A. Pileri, G. Maraini and F. Gavosto, Expl Cell Res. *20*, 654 (1960).
[3] F. Gavosto, A. Pileri and G. Maraini, Proc. 7th Congr. Europ. Soc. Haemat. London 1969; part II, p. 380 (1960).
[4] J. Brachet and P. Malpoix, Adv. Morphogenesis *9*, 263 (1971).
[5] J. Brachet and N. Hulin, Expl Cell Res. *59*, 486 (1970).
[6] Z. Darzynkiewicz, L. Bolund and N. R. Ringertz, Expl Cell Res. *55*, 120 (1969).
[7] J. Brachet and N. Hulin, Nature, Lond. *222*, 481 (1969).
[8] J. Brachet and A. Ficq, Expl Cell Res. *38*, 153 (1965).
[9] A. Pileri, P. Masera, N. Hulin and J. Brachet, Acta haemat. *48*, 89 (1972).