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 atrophy3. 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 increased³. 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.

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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 ³H-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 ³H-actinomycin binding were evaluated on bone marrow cells from 2 normal subjects. The in situ technique of Brachet and Fico 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. Malfoix, 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. Fico, Expl Cell Res. 38, 153 (1965).
- ⁹ A. PILERI, P. MASERA, N. HULIN and J. BRACHET, Acta haemat. 48, 89 (1972).

Uridine incorporation and actino	mycin binding: mear	grain counts (MGC)	in 2 normal human	bone-marrow specimens
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Marrow cells	Case 1		Case 2	
	Uridine (MGC)	Actinomycin (MGC)	Uridine (MGC)	Actinomycin (MGC
Myeloblasts	114.6	114.7	103	96.4
Promyelocytes	98	107.5	91.1	103.5
Neutr. myelocytes	25	73.8	24.9	72.5
Neutr. metamyelocytes	2.8	65.8	2.3	59.1
Neutr. granulocytes	1.3	61.5	0.9	53.6
Proerythroblasts	154.6	118.6	228	128
Bas. erythroblasts	71.9	70.3	94.7	93.5
Early poly.erythroblasts	10.1	62.7	9.7	55.4
Late poly.erythroblasts	3.2	53.7	3	51.4
Lymphocytes	1.6	39	0.8	44.6

acid (70:30) at 0 $^{\circ}\text{C}$ and then placed in a 5 $\mu\text{Ci/ml}$ $^{8}\text{H-}$ actinomycin solution (spec. activity: $6.5~{\rm Ci/m}M$) for 1 h at room temperature. They were next treated with 10 μg/ml unlabelled actinomycin and rinsed in running water overnight before preparation for autoradiographic processing in Ilford K2 emulsion, and stained with May-Grünwald-Giemsa. For uridine uptake, bone marrow diluted 1:1 with Hank's medium was incubated at 37°C for 1 h with 10 $\mu \text{Ci/ml}$ 5-T-uridine (spec. activity 3 Ci/m M). Preparation of the smears was followed by fixing in acetic Carnoy and autoradiographic processing 1-2. Grain counts were made for each cell type and in each case on a minimum of 50 cells for myeloblasts and proerythroblasts, or 200 cells for all other types. The mean grain count was then determined. When selecting the autoradiographic preparations, use was made of exposures (20 days) showing significant labelling even on the part of very mature erythroid and myeloid cells.

Results and discussion. The Table gives the mean grain counts for actinomycin D binding and uridine incorporation in myeloid and erythroid cells.

It can be seen that actinomycin D binding values decrease in function of increasing differentiation in both cell lines. This pattern is even more striking in the case of uridine incorporation. Here variations of the order of 100:1 between cells in different stages of differentiation have been noted, in contrast to the approximately 2:1 variations found for actinomycin binding values.

This disparity could be due to large differences in ribosomal RNA synthesis during the various stages of differentiation, while variations in genetic transcription, i.e. messenger RNA synthesis, might be much smaller. The latter is presumably evidenced by the ability of chromatin to bind actinomycin D, while it is clear that uridine incorporation will only permit evaluation of total RNA

activity; no distinction between ribosomal RNA synthesis and the lesser messenger RNA and transfer RNA syntheses has so far been possible with this method. In other words, nucleolar organiser DNA would be more active in immature nucleolated cells and this would result in intensive ribosomal RNA synthesis: this high activity would be detectable by measurement of uridine incorporation, but not by that of actinomycin D binding. These findings are in line with recent work on macromolecular synthesis during early embryonic development showing that the synthesis of the various kinds of RNA is not coordinated 4,10.

Riassunto. Nel midollo umano normale, parallelamente alla differenziazione cellulare delle linee mieloide ed eritroide, si osserva, per l'incorporazione dell'uridina nell'ARN, una diminuzione maggiore di quella della fissazione dell'actinomicina-D alla cromatina.

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Immunosuppressive Activity of Azathioprine in Experimental Infection of the Mouse with *Trichinella spiralis* (Nematoda)

Mice infected with the muscle stage of *Trichinella spiralis* characteristically expel the majority of adult worms from the intestine during the second week of the initial infection. This expulsion is due to a primary delayed hypersensitivity (cellular) reaction against the adults and a secondary inflammatory change in the intestinal tissue¹. This expulsion has been suppressed by various immunosuppressive compounds in various hosts². The effect of

azathioprine on *T. spiralis* has been reported only for the guinea pig². As a result, the objectives of this study were to investigate the efficacy of azathioprine against the

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¹ J. E. LARSH, N. F. WEATHERLY, H. T. GOULSON and E. F. CHAFFEE, J. Parasit. 58, 1052 (1972).

² J. P. Harley, Proc. helminth. Soc. Wash. 40, 242 (1973).