turbed development of these parts. The rhinencephalic deformations might affect emotional determinants of their behaviour.

One might argue that the widening of the telencephalic ventricles in mutants is due to increased pressure of the cerebrospinal fluid. However, the third and fourth ventricles appeared to be unaffected by this. In view of the fact that we did not observe any proliferation of the chorioid plexuses, nor any obstructions within the ventricular system, the problem of the ultimate cause of the distension of the lateral ventricles needs further investigation.

Résumé. Les souris hétérozygotes appartenant à la mutation «loop-tail» (*Lp*) présentent une hydrocéphalie interne du télencéphale. Jusqu'ici, cette anomalie ainsi que les torsions de la queue et l'oscillation de la tête qui l'accompagnent n'avaient pas été étudiés en détail. Nous avons constaté que par suite de l'élargissement des ventricules encéphaliques, les structures cérébrales qui les entourent sont réduites, déformées ou déplacées. Ces aberrations neuro-anatomiques jettent de la lumière sur les perturbations du comportement observées chez ces mutants.

J. H. F. VAN ABEELEN
and SYLVIA M. J. RAVEN

Genetics Laboratory, University of Nijmegen (The Netherlands), 3 July 1967.

Organogenesis: Prolonged Differentiation and Growth of Tooth Primordia on the Chick Chorio-Allantoic Membrane

The capacity for embryonic organ primordia to differentiate independently can be tested by isolation from the in situ environment and transplantation to an artificial milieu suitable for development. The chick chorio-allantoic membrane (CAM) provides a versatile host site for the maintenance and further differentiation of numerous embryonic organs[1-3]. One such organ, the tooth organ primordium, presumably arises as a result of epithelio-mesenchymal interactions. Upon entering the terminal stages of early postnatal differentiation the odontogenic cells initiate the synthesis of specific species of fibrous proteins which are involved in the formation of extracellular organic matrices.

Several investigations have indicated how rodent molar xenografts, transplanted from the host organism and grown on the CAM, advanced further developmentally than those grown in vitro[4-7]. Usually, transplantation of organ primordium on the CAM can be accomplished so that the graft is maintained for 10 continuous days of incubation. This study, however, has prolonged that period of development by culturing tooth organ primordia for many more days of uninterrupted incubation by re-transplantation to new CAM sites. The utility of the re-transplantation methodology has been successfully used and demonstrates significant potential for prolonged investigation of differentiation and growth.

Materials and methods. Donor tissues. Embryos were obtained by caesarian section from Wistar rats. The morning of the vaginal plug discovery was counted as day 0. Maxillary molar organ primordia were dissected from 19 day embryos (Figure 1). Dissection of the molar primordium was initially accomplished by procedures previously described[8]. We modified this technique to exclude any intermediate treatment of the donor tissues after observing an increased graft survival as a result of placing the excised primordium immediately on the CAM.

Controls were prepared for each litter of 19 day embryos to establish the chronological verification for the stage of development. Randomly selected maxillae were fixed and serially sectioned for this purpose.

Method of CAM-grafting. Austra White (Australop ♂ × White Leghorn ♀) embryonated hen's eggs were incubated for 8 days at 37.5 °C. On the eighth day of incubation the major branches of the vitelline blood vessels were determined by candling and appropriately recorded. The hosts were prepared for CAM grafting by procedures previously described[9]. The shell surface was cleansed with alcohol prior to cutting a 5 × 5 mm window above the region previously determined for the site of grafting. Upon elevation of the shell window, the adjacent shell membrane was incised and the underlying chorionic epithelial layer was exposed. Following explantation the shell was re-positioned and sealed with paraffin. Each host was returned to the incubator with the graft facing downward for the first 24 h of incubation; a procedure which allowed the weight of the host to facilitate the adaptation of the graft. Thereafter, each egg was turned twice daily and candled. The viability of the chick embryo was used as the criterion for assessing graft survival.

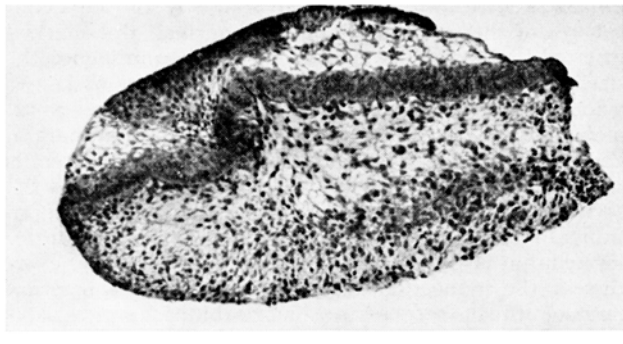

Fig. 1. The molar primordium explant immediately following excision from the donor 19-day rat embryo. Stained with hematoxylin and eosin. × 65.

[1] C. GROBSTEIN and E. ZWILLING, J. exp. Zool. *122*, 259 (1953).
[2] E. ZWILLING, Transplantn Bull. *6*, 115 (1959).
[3] A. MOSCONA, Expl Cell Res. *22*, 455 (1961).
[4] S. GLASSTONE, J. Anat. *88*, 392 (1954).
[5] A. N. STUDITSKY, Anat. Anz. *83*, 304 (1939).
[6] M. F. HAY, Archs oral Biol. *3*, 86 (1961).
[7] H. C. SLAVKIN and L. A. BAVETTA, Int. Ass. dent. Res. Proc. 177 (1967).
[8] W. LEFKOWITZ and D. F. MARDFIN, J. dent. Res. *33*, 201 (1954).
[9] H. C. SLAVKIN and L. A. BAVETTA, Archs oral Biol., in press.

Following 10 days of incubation, surviving grafts were excised from the CAM and re-transplanted to a second group of 8 day embryonated eggs. These procedures were carried out as previously described. After the second 10 day period of incubation (20 days of continuous cultivation), surviving grafts were excised and re-transplanted to a third group of 8 day hosts. The experiments were terminated at the end of the sixth day in the third period of incubation since few grafts survived.

The findings to be reported are based upon more than 1055 total CAM grafts. This study extended through 3 seasons of the year so that seasonal variations in the incubation and source of the host eggs were considered to be minimal.

Histological treatment. The controls and surviving grafts were fixed in Hollande-Bouin's solution. Decalcification of these tissues was not performed. The tooth germs were embedded in paraffin and sections were cut at 5 μ for all tissues except those cultivated for 26 days. The latter xenografts were sectioned at 15 μ to compensate for the lack of decalcification. Both hematoxylin and eosin or periodic acid-Schiff were used to stain the sections.

Xenograft odontogenesis after 10 days on the CAM. After grafting the donor organ primordium on the CAM of an 8 day host and subsequent incubation for 10 days, gross observations revealed a plexus of capillaries surrounding the donor tissues. It is possible to explant 2 grafts on the same host CAM, however, one xenograft per host was used for all re-transplantation experiments. The data summarized in the Table reports the survival times of 19 day embryonic xenogeneic molar grafts on the chick CAM. Gross examination of the grafts prior to excision and re-transplantation revealed the formations of cusp elevations and the general absence of peripheral 'growing-out' or 'flattening' of the odontogenic apparatus. The general gross morphological observations suggested further development of the molar xenografts.

Developmental variations, ranging from no extracellular matrix formation to the appearance of dentin and enamel, were frequently observed. In most xenografts after 10 days of cultivation, numerous blood vessels were noted in the CAM adjacent to the grafts. These vessels appeared in intimate association with the primordia suggesting the host's participation in the success of the xenograft's adaptation. The chorionic epithelium, immediately in contact with the graft, did not react with the adjacent odontogenic cells. Both host and graft epithelium appear to have retained their individual cellular integrity.

Xenograft odontogenesis after 26 days on the CAM. As shown in the Table very few xenografts survived 26 days of continuous cultivation in the avian environment. All surviving grafts in this group did not reach the same stage of organ development. Most representative of the prolonged differentiation and growth, however, was the degree of characteristic rodent molar crown morphology attained without aberration (Figure 2). The rodent maxillary first molar has 3 buccal cusps and 2 palatal cusps which do not fuse until crown development is complete near the tenth day postnatal age. Our surviving xenografts demonstrated remarkable increases in over-all size and morphodifferentiation. The rodent cusp tips are not covered with enamel in vivo in that they have a characteristic dentin occlusal region with a discrete junction between the adjacent enamel tissues. The dental papilla, now the dental pulp, functioned to maintain the dentin matrix in the form of odontoblasts secreting collagen, and an amorphous cell population of fibroblast-like cells serving a supportive and nutritive function. Continuous fibers can be seen to extend from the odontoblasts and pulpal tissues, across the dentin matrices, to the lamina separating the dentin and enamel. Similar fibrous proteins extrude from the ameloblasts to form the enamel extracellular organic matrix. The 26 day xenografts therefore suggested that normal histodifferentiation, morphodifferentiation, and mineralization had occurred.

Xenograft mortality. By the termination of these experiments, 26 days of incubation, a 97% mortality was consistently observed. The donor primordia, rather than host, incubation temperature and humidity, season of the year, or surgical trauma, was assumed to be the primary variable with respect to graft mortality.

Discussion. Besides the principle of induction, reflecting epithelio-mesenchymal interaction, self-organization may be a basic intrinsic property, within the transplanted rudiment, responsible for continued differentiation and growth[10]. The morphological and developmental results reported in this study indicate the embryonic rat molar primordia will grow and differentiate for 26 continuous days as xenografts cultivated on the chick CAM. The

Survival times of 19 day embryonic rat xenogenic molar grafts on chick CAM

No. embryos per group	No. molar primordia excised	No. sham-operations	No. surviving sham-operated	No. hosts with surviving grafts: 10 (days)	20	26
25	50	8	8	25	10	2
31	62	8	6	31	15	1
30	60	8	6	30	15	2
41	82	10	9	45	20	3
35	70	6	6	40	22	3
32	64	10	9	32	16	2
37	72	4	4	37	20	2
20	40	10	10	15	6	0
50	100	20	17	50	26	4

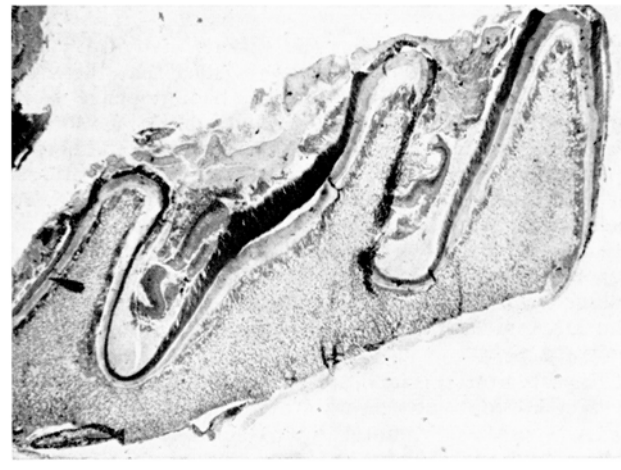

Fig. 2. Longitudinal (mesiodistal) section through a complete crown of a xenograft cultivated for 26 days on the CAM. × 65.

[10] J. Holtfreter and V. Hamburger, in *Analysis of Development* (1955), p. 230.

avian environment, a passive or indifferent environment for odontogenesis, can be considered not to have furnished appropriate cues for further organogenesis of the completely isolated primordium. It is therefore concluded that the prolonged differentiation and growth of the excised primodrium, cultivated in an indifferent environment, reflects an intrinsic, intra-organ, self-perpetuating potential.

The observations reported show that during the cultivation of the xenografts on the chick CAM for 10 days incubation, normal morphodifferentiation was observed, albeit at a much slower rate than that occurring in vivo[11,12]. The most favorable differentiation and growth patterns have been reported when 19 day rodent molar primordia are cultivated in vitro or as transplants in vivo[4,8,13]. The organ culture procedures employed in this study agreed with these other investigations in that embryonic rat excised molars were inherently committed to form a tooth.

The potential of the chorionic epithelium of the chick CAM to actively interact with the grafted tissues, or to merely be a passive support and nutritive component, was considered in this study. It was concluded that the host chorionic epithelium, immediately adjacent to the xenograft, did not interact with the transplanted tissues, but rather provided an exquisite nutritive or supportive function.

Numerous in vitro, allograft, and xenograft studies have not been able to show a time schedule of developmental events approximating that seen in utero or in the postnatal animal. Similarly, our studies did not demonstrate a chronology comparable to that in vivo. Completely excised 19 day embryonic donor tissues require 26 days in an indifferent environment to achieve approximately 13 days of in vivo organgenesis. The surgical excision and resulting trauma, the period of time in which the donor primordium was removed from an actively provided nutritional environment, the drastic temperature changes during the CAM grafting procedures, and the changes in the oxygen pressures, are variables which give credibility for the time lag observed. Nevertheless, it seems valid to interpret the data to indicate that the chick CAM-grafting procedure offers an ideal method for cultivating organ primordia and obtaining advanced differentiation and growth in a predictable fashion[14].

Zusammenfassung. Es wird gezeigt, dass die Molarenanlagen von einem Nager auf dem Allantochorion des Hühnchens sich herkunftsgemäss weiterentwickeln und echte Zähne ausbilden.

H. C. SLAVKIN[15] and L. A. BAVETTA[16]

University of Southern California School of Dentistry, Department of Biochemistry Los Angeles (California 90007, USA),
4 August 1967.

[11] W. LEFKOWITZ, C. F. BODECKER and D. F. MARDFIN, J. dent. Res. *23*, 749 (1953).
[12] I. SCHOUR and M. MASSLER, in *The Rat in Laboratory Investigation* (1962), p. 106.
[13] H. S. FLEMMING, Oral Surg. *8*, 3 (1955).
[14] The authors wish to especially recognize the generous and stimulating guidance given by Dr. E. L. COOPER (Department of Anatomy, University of California at Los Angeles) during the progress of this study. The authors are indebted to Mr. C. TETREAULT, Mrs. IDA IKHURST and Mrs. ZOYA TRIROGOFF for their invaluable technical assistance, and to Mrs. JOANNE LEYNNWOOD for her help in the preparation of this manuscript. This program of research was supported in parts by research grants from the National Institute for Dental Research (No. DE 02471-01 and No. DE 01177-08).
[15] Postdoctoral trainee grant No. 5-T01 DE-00094-05 from the U.S. Public Health Service, Institute of Dental Research.
[16] Recipient of Career Award No. 1-K6-DE-6083-03 from the U.S. Public Health Service, Institute of Dental Research.

Effects of Certain Hydroxamic Acids on Virus Replication

Recent work concerning the actions of hydroxyurea (HU) on virus development has revealed that the drug reduces the yield of infectious T_4 bacteriophage from infected cells of *Escherichia coli*[1] and inhibits the formation of mature infectious particles of vaccinia[2] and herpes simplex[3] viruses with no apparent effect on the synthesis of viral protein. In each case the aberrations noted were believed to be attributable to a reduction by the drug of the rate of viral DNA synthesis, similar to the previously reported effects on bacterial[4,5], mammalian[6,7], and echinoderm[8] test systems. In addition, HU interferes with the DNA metabolism of BHK-21 cells transformed by polyoma virus[9].

Reports from this laboratory have described actions of certain HU derivatives and other hydroxamic acids on DNA synthesis. Oxamyl hydroxamic acid (OHA)[10], salicyl hydroxamic acid (SHA)[11], and acetoxyoxamide (AOA)[12] selectively suppress thymidine incorporation into mammalian and/or bacterial test systems with no appreciable diminution of the rate of RNA or protein synthesis. Considering potential applications of agents which may reduce the rate of synthesis of viral DNA without substantially depressing the formation of the antigenic viral protein, the following preliminary study was initiated to determine if these HU derivatives and SHA influence the replication of the *E. coli* bacteriophage, and to contrast any observed activity with that of HU.

[1] W. MARGARETTEN, C. MORGAN, H. S. ROSENKRANZ and H. M. ROSE, J. Bacteriol. *91*, 823 (1966).
[2] H. S. ROSENKRANZ, H. M. ROSE, C. MORGAN and K. C. HSU, Virology *28*, 510 (1966).
[3] S. NII, C. MORGAN, H. M. ROSE and H. S. ROSENKRANZ, Fedn Proc. Fedn Am. Socs exp. Biol. *25*, 311 (1966).
[4] G. R. GALE, S. M. KENDALL, H. H. MCLAIN and S. DUBOIS, Cancer Res. *24*, 1012 (1964).
[5] H. S. ROSENKRANZ and J. A. LEVI, Biochim. biophys. Acta *95*, 181 (1965).
[6] C. W. YOUNG, S. HODAS and J. J. FENNELLY, Proc. Am. Ass. Cancer Res. *5*, 71 (1964).
[7] G. R. GALE, Biochem. Pharmac. *13*, 1377 (1964).
[8] M. L. MURPHY and S. CHAUBE, Cancer Chemother. Rep. *40*, 1 (1964).
[9] R. D. POLLAK and H. S. ROSENKRANZ, Cancer Res. *27*, 1214 (1967).
[10] G. R. GALE, Cancer Res. *26*, 2340 (1966).
[11] G. R. GALE, Proc. Soc. exp. Biol. Med. *122*, 1236 (1966).
[12] G. R. GALE, J. natn. Cancer Inst. *38*, 51 (1967).