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.

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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).
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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).

HU, OHA, AOA, and SHA were obtained from Hynes Chemical Research Corporation, Durham, N.C., USA. *E. coli* B and its T_4 bacteriophage were from the American Type Culture Collection. Dose-response relationships of the virus to various concentrations of each compound were assessed following a 10 min absorption interval in synthetic medium[13] with the virus in a 10:1 multiplicity. Upon removal of unabsorbed virus by centrifugation and resuspension 3 times, the infected bacteria were resuspended to the original volume in the synthetic medium containing each filter-sterilized compound. After 90 min at 37 °C, chloroform was added and the tubes were stored overnight at 5 °C. Following centrifugation the amount of infectious virus in the supernatant solution was determined by the double agar layer technique[14]. Plaques were counted independently by 2 operators and the average values were calculated.

Effects of hydroxyurea (HU), oxamyl hydroxamic acid (OHA), salicylhydroxamic acid (SHA), and acetoxyoxamide (AOA) on the yield of infectious T_4 bacteriophage from *Escherichia coli* B.

Compound	Concentration (M)	% inhibition
HU	10^{-5}	3
HU	10^{-4}	13
HU	10^{-3}	63
HU	10^{-2}	71
OHA	10^{-5}	21
OHA	10^{-4}	70
OHA	10^{-3}	93
OHA	10^{-2}	97
SHA	10^{-5}	34
SHA	10^{-4}	49
SHA	10^{-3}	89
SHA	10^{-2}	96
AOA	10^{-5}	24
AOA	10^{-4}	33
AOA	10^{-3}	43
AOA	10^{-2}	95

All values are averages of 3 experiments.

The Table shows that at $10^{-5}M$ and $10^{-4}M$, OHA, SHA, and AOA retarded virus replication and were somewhat more active than HU. At $10^{-3}M$ AOA was less active than HU, but the 3 former compounds induced 95% inhibition or greater at $10^{-2}M$ as compared with 71% inhibition obtained with HU at the same concentration. OHA was the most active compound, yielding 70% inhibition at $10^{-4}M$. Further evaluation of these agents and other hydroxamic acids in a variety of virus systems consequently seems clearly indicated[15].

Résumé. L'acide oxamyl-hydroxamique, l'acide salicylhydroxamique et l'acétoxyoxamide inhibent la reproduction du bactériophage T_4 dans l'*Escherichia coli* B. L'activité de chacun de ces composés était plus grande que celle de l'hydroxyurée dans le même système d'essai. De ce fait, une évaluation plus poussée de ces 3 agents ainsi que d'autres acides hydroxamiques dans une série de systèmes d'essai sur les virus paraît clairement indiquée.

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11 September 1967.

[13] B. D. Davis and E. S. Mingioli, J. Bacteriol. *60*, 17 (1950).
[14] M. H. Adams, *Bacteriophages* (Interscience, New York 1966).
[15] Aided by grant No. GM-13958 from the National Institutes of Health, United States Public Health Service.

Some Aspects of Novobiocin Action on *Escherichia coli* and *Staphylococcus aureus*

Novobiocin, an antibiotic originally known as 'streptonivicin', was described in 1956[1]. It is usually employed in the form of its monosodium salt, and is active mainly against Gram-positive bacteria.

In contrast to the many investigations carried out on the mechanisms whereby other antibacterial substances exert their effect, relatively few reports have been made as to the exact nature of the action of novobiocin. The following are among the effects observed in novobiocin-treated bacteria: (a) an inhibition of cell wall, protein and nucleic acid syntheses[2]; (b) induction of spheroplasts[3] (but compare with Wishnow et al.[2]); (c) an increase in the permeability of *Escherichia coli*[4]; (d) an intracellular deficiency of magnesium ions[5], although contrary evidence, viz. that the antibiotic and Mg^{++} do not form a complex, has since been produced[6,7]; (e) an inhibition of deoxyribonucleic acid (DNA) synthesis prior to any inhibition of cell wall, protein and ribonucleic acid (RNA) syntheses[8].

The following experiments describe the effects of novobiocin on viable and total counts and turbidity of growing and non-growing cultures of *E. coli* NCTC 9001 and *Staphylococcus aureus* NCTC 6571. These organisms were grown overnight at 37 °C in 40 ml of nutrient broth No. 2 and nutrient broth (both from Oxoid Laboratories, Ltd., London, England) respectively. The cultures were centrifuged at 2500 rpm for 10 min, the supernatant fluids removed, and the pellets washed twice in sterile water. The suspensions were finally adjusted to contain about 10^8 viable cells/ml. Novobiocin monosodium, B.P., was purchased from Merck, Sharpe and Dohme, Ltd., Hoddes-

[1] C. G. Smith, A. Dietz, W. T. Sokolski and G. M. Savage, Antibiotics Chemother. *6*, 135 (1956).
[2] R. M. Wishnow, J. L. Strominger, C. H. Birge and R. H. Threnn, J. Bact. *89*, 1117 (1965).
[3] F. E. Hahn, Cited by K. McQuillen, in *The Bacteria* (Eds I. C. Gunsalus and R. Y. Stanier; Academic Press, New York 1960), vol. 1.
[4] T. D. Brock and M. L. Brock, Archs Biochem. Biophys. *85*, 176 (1959).
[5] T. D. Brock, Science *136*, 316 (1962).
[6] P. J. Niebergall, D. A. Hussar, W. A. Cressman, E. T. Sugita and J. T. Doluisio, J. Pharm. Pharmac. *18*, 729 (1966).
[7] A. Morris, A. D. Russell and I. L. Thomas, Experientia *23*, 244 (1967).
[8] B. H. Smith and B. D. Davis, J. Bact. *93*, 71 (1967).