to 1.0 × 10^8 PFU per culture 72 hours later. The type 2 adenovirus had acquired the ability to replicate in green-monkey kidney cells after interaction in these cells with the monkey-adapting component present in the adeno 7 (M) stock. This growth capability was also demonstrated (Table 2) by the fact that plaques formed in green-monkey kidney cells after inoculation of adeno 2 (M). Growth analyses in monkey cells (*8*) have revealed that the monkey-adapting component does not replicate in the absence of helper adenovirus, a situation similar to that of the PARA (defective SV40) component carried by other adenovirus populations (*6*).

Thus plaque formation in green-monkey kidney cells by a monkey-adapted strain of adenovirus 7 requires the interaction of two particles, both of which are neutralized by adenovirus antiserum. Addition of nonadapted adenovirus type 2 as the helper adenovirus results in adenovirus 2 progeny, which carry the determinant or determinants of the monkey-adapting component and are able to replicate in green-monkey kidney cells. These results demonstrate that not every monkey-adapted adenovirus carries information which codes for the synthesis of SV40 T antigen, for the adenovirus populations carrying the monkey-adapting component do not induce SV40 T antigen. However, the monkey-adapting component adenovirus being studied may be carrying SV40 genetic information defective not only for viral structural determinants, but also for the tumor antigen marker.

Currently the origin of the information in the monkey-adapting component is unknown. The monkey-adapting component is the second example of a foreign determinant being carried by adenoviruses, the first example being known SV40 determinants. In both instances, acquisition of the foreign determinants enables the adenovirus to replicate in green-monkey kidney cells.

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References and Notes

1. G. T. O'Conor, A. S. Rabson, I. K. Berezesky, F. J. Paul, *J. Nat. Cancer Inst.* **31**, 903 (1963); J. S. Butel and F. Rapp, *J. Bacteriol.* **91**, 278 (1966); L. A. Feldman, J. S. Butel, F. Rapp, *ibid.*, p. 813. (1966).
2. W. B. Beardmore, M. J. Havlick, A. Serafini, I. W. McLean, Jr., *J. Immunol.* **95**, 422 (1965); J. M. Easton and C. W. Hiatt, *Proc. Nat. Acad. Sci. U.S.* **54**, 1100 (1965); K. Schell, W. T. Lane, M. J. Casey, R. J. Huebner, *ibid.* **55**, 81 (1966); A. M. Lewis, Jr., K. O. Prigge, W. P. Rowe, *ibid.* **55**, 526 (1966).
3. R. J. Huebner, R. M. Chanock, B. A. Rubin, M. J. Casey, *Proc. Nat. Acad. Sci. U.S.* **52**, 1333 (1964); F. Rapp, J. L. Melnick, J. S. Butel, T. Kitahara, *ibid.* **52**, 1348 (1964); W. P. Rowe and S. G. Baum, *ibid.* **52**, 1340 (1964); J. S. Butel and F. Rapp, *J. Bacteriol.* **91**, 278 (1966).
4. A. M. Lewis, Jr., S. G. Baum, K. O. Prigge, W. P. Rowe, *Proc. Soc. Exp. Biol. Med.* **122**, 214 (1966).
5. J. L. Melnick, H. D. Mayor, K. O. Smith, F. Rapp, *J. Bacteriol.* **90**, 271 (1965).
6. J. S. Butel and F. Rapp, *ibid.* **91**, 278 (1966); A. Boeyé, J. L. Melnick, F. Rapp, *Virology* **28**, 56 (1966); W. P. Rowe and S. G. Baum, *J. Exp. Med.* **122**, 955 (1965).
7. F. Rapp, J. S. Butel, J. L. Melnick, *Proc. Nat. Acad. Sci. U.S.* **54**, 717 (1965); W. P. Rowe, *ibid.*, p. 711.
8. J. S. Butel and F. Rapp, in preparation.
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Liver Cancer: Neonatal Estrogen Enhances Induction by a Carcinogen

Abstract. *A single injection of 100 micrograms of estradiol benzoate into newborn rats was followed after weaning by dietary treatment with one of two dosages of the carcinogen N-hydroxy-N-2-fluorenylacetamide. Autopsies 26 weeks later showed a higher incidence of liver cancer in male and, particularly, female rats injected with hormone than in controls. The weights of livers were greater but gonads were smaller in size in the estradiol groups. Endocrine and possibly central-nervous-system factors may play roles in formation of liver tumors.*

Injection of hormones into infant rats has remarkable effects, expressed later in the adults by altered behavioral and physiological responses. The mechanisms involved, especially in regard to sexual activity, are apparently mediated by the central nervous system (*1*).

This technique has now been used to determine the role of hormones and underlying pituitary-hypothalamic controlling elements in the carcinogenic process—for the study of tumor formation in the liver (*2*). The liver of male rats is generally more susceptible to carcinogenic aromatic amine derivatives such as *N*-2-fluorenylacetamide and its active metabolite *N*-hydroxy-*N*-2-fluorenylacetamide (*3*), so induction of liver cancer was studied in male and female rats given a single dose of estrogen at birth.

Rats of the Fischer strain were injected subcutaneously within 24 hours of birth with 100 μg of estradiol benzoate suspended in 0.03 ml of a 1-

Table 1. Effect of a single neonatal injection of estradiol benzoate on physiological parameters and on formation of liver tumors. N-OH-FAA, *N*-hydroxy-*N*-2-fluorenylacetamide; upon weaning at 4 weeks of age the groups on 80 ppm received the carcinogenic diet for 20 weeks and then control diet for 6 weeks; groups on 160 ppm carcinogen were so fed for 16 weeks and then placed on control diets for 10 weeks. Lesions in livers were graded by the system of Reuber (*6*). Female rats in groups receiving 80 ppm N-OH-FAA and no hormone had more foci of hyperplasia and fewer of the more advanced hyperplastic nodules than the groups on 80 ppm treated with estrogen.

Conc. N-OH-FAA (ppm)	Estradiol	Rats (No.)	Weight*			Liver (rats, No.)	
			Body (g)	Liver (g/100 g)	Gonad (g), ovary (mg)	Hyperplasia	Cancer
			Males				
0	Yes	10	284 ± 8	2.54 ± 0.10	1.54 ± 0.25	0	0
80	Yes	10	249 ± 9	5.82 ± .40†	1.47 ± .19	2	8
80	No	10	254 ± 6	4.76 ± .26†	2.54 ± .07	5	5
160	Yes	15	236 ± 4	7.81 ± .44	1.51 ± .19	2	13
160	No	11	248 ± 4	7.46 ± .41	2.76 ± .03	1	10
			Females				
0	Yes	12	211 ± 5	2.51 ± 0.06	26.2 ± 3.2	0	0
80	Yes	13	206 ± 3	3.87 ± .14†	23.1 ± 1.8	13	0
80	No	8	178 ± 2	2.84 ± .07†	51.6 ± 2.5	8	0
160	Yes	22	185 ± 2	5.30 ± .20†	24.9 ± 1.6	13	9
160	No	16	173 ± 3	3.58 ± .10†	54.0 ± 1.6	16	0

* Mean and S.E.; the larger standard error in the liver weights in some groups reflects the more or less extensive and advanced development of lesions. † $P < .05$, by Student's *t*-test, between appropriate groups shown (estradiol "yes" or "no" at each dosage).

percent aqueous solution of gelatin (*4*); control rats received the vehicle alone. Upon weaning, the animals were fed diets containing 0, 80, or 160 ppm of *N*-hydroxy-*N*-2-fluorenylacetamide (*5*). After 26 weeks the rats were killed and carefully autopsied; selected organs were weighed and fixed for histopathological examination.

Male and female rats treated once at birth with estrogen had atrophic gonads; the females also had smaller uteri. The livers of the control animals not treated with carcinogen and injected with hormone were lower in weight than is customary in Fischer rats (2.9 to 3 g per 100 grams of rat; Table 1). The incidence of hepatoma in male rats fed 160 ppm of carcinogen was similar in groups injected with estrogen or vehicle. At the lower dosage of carcinogen, however, the single neonatal dose of hormone resulted in more rats with heavier livers and with hepatoma than among the controls. In female rats the effect was accentuated, especially at the higher carcinogen dosage; the hormone-treated groups had higher liver weights and cancer incidence at the 160-ppm carcinogen dosage, while at the lower dosage the precancerous lesions were more advanced.

It appears, therefore, that neonatal injection of hormone exerts a powerful effect on both the direct sexual behavior of rats (*1*) and the physiological and pathological processes subject to hormonal control mechanisms—including the induction of liver cancer. Detailed analysis of the complex elements participating, such as function and differentiation of the components of the endocrine system, in relation to the hormonal requirements for liver carcinogenesis may contribute to both psychophysiology and knowledge of cancer.

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References and Notes

1. R. E. Whalen and R. D. Nadler, *Science* **141**, 273 (1963); R. E. Whalen, *J. Comp. Physiol. Psychol.* **57**, 175 (1964); S. Levine and R. Mullins, Jr., *Science* **144**, 185 (1964); H. H. Feder and R. E. Whalen, *ibid.* **147**, 306 (1965); S. Levine, *Sci. Amer.* **214**, 84 (1966); ——— and R. F. Mullins, Jr., *Science* **152**, 1585 (1966).
2. R. L. Noble, in *The Hormones*, G. Pincus, K. V. Thimann, E. B. Astwood, Eds. (Academic Press, New York 1964), vol. 5, pp. 559–695; D. B. Clayson, *Chemical Carcinogenesis* (Little, Brown, Boston, 1962), chap. 13, 14; N. I. Lazarev, *Dyshormonal Tumors* (Consultants Bureau, New York, 1966); A. Lacassagne, in *Hormonal Steroids, Biochemistry, Pharmacology and Therapeutics: Proc. First Intern. Congr. Hormonal Steroids*, L. Martini and A. Pecile, Eds. (Academic Press, New York, 1965), vol. 2, pp. 379–90; C. Théret, *Rev. Intern. Hepatol.* **12**, 1 (1962).
3. E. C. Miller, J. A. Miller, H. A. Hartmann, *Cancer Res.* **21**, 815 (1961); J. H. Weisburger, S. R. Pai, R. S. Yamamoto, *J. Nat. Cancer Inst.* **32**, 881 (1964); E. K. Weisburger and J. H. Weisburger, *Advan. Cancer Res.* **5**, 331 (1958). *N*-Hydroxy-*N*-2-fluorenylacetamide is also called *N*-hydroxy-2-acetylaminofluorene. *Chemical Abstracts* renders the name *N*-2-fluorenylacetohydroxamic acid.
4. Vehicle described by G. Pietra, H. Rappaport, P. Shubik, *Cancer* **14**, 308 (1961).
5. Y. Shirasu, P. H. Grantham, R. S. Yamamoto, J. H. Weisburger, *Cancer Res.* **26**, 600 (1966).
6. M. Reuber, *J. Nat. Cancer Inst.* **34**, 697 (1965); grading in order of increasing severity: 1, focus of hyperplasia, area of hyperplasia, nodule of hyperplasia (all under "Hyperplasia"); 2, small hepatoma and hepatoma (under "Cancer").
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Inhibition of the Carotid Sinus Reflex by Stimulation of the Inferior Olive

Abstract. *A projection of nerve fibers from rostral brainstem areas, which produce pressor responses and tachycardia, terminates in the inferior olive. Electrical stimulation of the olive in the cat produces no cardiovascular response but inhibits the depressor component of the carotid sinus reflex.*

Other than as a source of afferent input to the cerebellum, the inferior olive has been considered a nearly "nonfunctional" structure, despite its size and interesting morphology. The afferent projections to the inferior olive from other central neural structures (*1*), as well as the systematic projection from the inferior olive to the cerebellum (*2*), and the olive's status as the font of the olivospinal tract are well known anatomically. These connections, however, account for only an ill-defined role in motor coordination.

Surprisingly, anatomical study of the autonomic pathways in the brainstem included a projection to the medial portion of the olive from various diencephalic and midbrain structures in rats (*3*), cats, and dogs (*4*). The common feature of these disparate structures is that electrical stimulation of them uniformly results in large sustained increases in arterial pressure and tachycardia. Although stimulation of the portion of the olive receiving these projections might consequently be expected to have cardiovascular sequelae, stimuli localized to this area produced little or no change in cardiovascular function.

The consistent anatomical projection from cardiovascularly responsive areas in the more rostral portions of the brainstem can be reconciled with the absence of such responses to stimulation of the inferior olive, by postulating that the olive provides an inhibitory input to a cardiovascular reflex. The carotid sinus reflex immediately comes to mind. It is known that large sustained pressor responses accompanied by tachycardia are elicited by exercise or emotion as well as by diencephalic stimulation. Why the powerful carotid sinus reflex does not curtail this pattern of responses has never been explained.

The hypothesis that the "silent" effect of inferior olive stimulation is inhibitory to the carotid sinus reflex was tested in the following manner.

Ten cats were anesthetized with α-chloralose in water (40 mg/kg body weight), administered intraperitoneally. Femoral arterial blood pressure was recorded on a polygraph. The carotid arteries were exposed bilaterally, and loops of heavy suture were placed around them although the arteries were not occluded. A tracheal cannula was inserted. The cats were placed in a stereotaxic instrument, and the medial portion of the cerebellum was exposed in preparation for the insertion of stimulating electrodes into the medulla. The loops of suture around the arteries were connected to the shaft of a synchronous clock motor so that application of current to the motor stretched the arteries until the tissue resistance stalled the motor, thereby maintaining a constant pull on the arteries. Stretching of the carotids in this fashion gave a consistently repeatable bradycardia and a decrease in blood pressure.

After several control sinus reflex responses were obtained in this manner a coaxial stimulating electrode was stereotaxically placed in the brainstem in the area ranging from posterior 9 to 13, and 0.5 to 1.5 mm off the midline. The dorsal-ventral dimension