sites causes an elevation of IgE levels during childhood.

A compensation of IgA by IgE early in childhood appears an attractive hypothesis because it provides also an explanation for the existance of this class of immunoglobulins as it is difficult to imagine the evolution of a protein for which no useful function can be established. Indirect evidence of an interrelation between IgA and IgE is provided by the fact that atopic disease occurs more frequent in IgA deficient than in normal individuals[9,10].

JOHANSSON et al.[11] found higher levels of IgE in Ethiopian children with verified infections with *Ascaris lumbricoides* than in children with a negative stool. However, in a study in California, no uniform elevating of the serum IgE level was found in individuals with ascariasis[12]. It appears unlikely, therefore, that in the present study the elevated levels in children did result from ascariasis alone inasmuch as infestation occurs also in adults[13]. The present data do not permit to establish to what extent the high IgE levels in children might result from ascariasis and to what extent it might represent a compensation for the relatively low IgA levels but likely both factors contributed to the elevated IgE. The large difference between Black and White children might in part also result from a difference in *Ascaris* infestation in addition to a racial difference as it was found for the other classes of immunoglobulins[2]. The data clearly show that age of the individual should be considered in studies on IgA and IgE levels.

[9] H. S. KAUFMAN and J. R. HOBBS, Lancet *2*, 1061 (1970).
[10] D. P. SCHWARTZ and R. H. BUCKLEY, New Engl. J. Med. *284*, 513 (1971).
[11] S. G. O. JOHANSSON, T. MELLBIN and B. VAHLQUIST, Lancet *1*, 1118 (1968).
[12] M. BAZARAL, H. A. ORGEL and R. N. HAMBURGER, Clin. exp. Immun. *14*, 117 (1973).
[13] G. M. JEFFERY, K. O. PHIFER, D. E. GATCH, A. J. HARRISON and J. C. SKINNER, Publ. Hlth. Rep., Wash. *78*, 45 (1963).

Failure of SV40-Transformed Lizard Cells to Induce Tumors in Autogeneic or Allogeneic Hosts

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Summary. Lizard cells from the tails of geckos were readily morphologically and antigenically transformed in vitro by SV40 virus. Neither autografts of these cells nor allografts of SV40 transformed gecko embryo cells produced tumors in animals under observation from 1 to 3 years.

Recently we reported that poikilothermic cells from two species of lizards, *Gekko gecko* and *Eublepharis maculatis*, could be cultivated in vitro and both morphologically and antigenically transformed by SV40[3,4]. To determine if these cells were also malignantly transformed, transplantation experiments were performed using the leopard gecko, *Eublepharis maculatis*. Since inbred lizards were not available, implants of transformed cells were made into allogeneic hosts and also into autogeneic hosts whose tail tissue had been cultured in vitro and transformed by SV40 virus. This report gives the results of these experiments.

Material and methods. The Leopard geckos, *Eublepharis maculatis*, used in this study were bred and reared in captivity. SV40 (RH911) propagated in CV-1 cells had a titer of 2×10^8 plaque forming units per milliliter (PFU/ml) in CV-1 cells. Assay for the SV40 T antigen was performed by the indirect immunofluorescence method[5].

Cell lines were derived from young adult geckos' (less than 6 months old) autotomized tail tissue. After removal of the epidermis, muscle and connective tissue were minced and dispersed with a trypsin-versene solution 0.25% trypsin and 0.1% versene in Ca^{2+} and Mg^{2+}—free PBS at room temperature (22–25 °C) for 20 min. The digest was filtered, pelleted and planted in flasks in Eagle's basal medium containing 10% fetal calf serum (BME-FCS 10) at 30 °C. The origin and methods of cultivation of the gecko embryo cell line, GE-1 and its transformation by SV40 have been reported elsewhere[2].

For transplantation, cells were dispersed with trypsin-versene solution, counted, pelleted, and resuspended in less than 0.5 ml of BME-FCS-10. GE-1 cells transformed by SV40 were used for allografts. Animals were inoculated either subcutaneously in the leg or intraperitoneally. In some animals non transformed cells at the same passage level were inoculated in the opposite leg to serve as controls.

Results. Cell cultures from the tail tissue of 4 animals were infected in suspension at high multiplicity at their first passage (3rd passage for T-8) with SV40 virus which produced little or no cytopathic effect. The SV40 infected cells and noninfected cells of the same culture that served as controls were subcultivated at about weekly intervals and monitored for the presence of SV40 T

Table I. Percentage of gecko cells exhibiting SV40 T antigen

Cell line	Virus multiplicity of infection	Cell passages after infection									
		1	2	3	4	5	6	7	8	17	18
T1	127	2			14	[a]		100			
T4	160	0		6		98[a]			100		
T5	480	[a]	91							100	
T8	80	4			12		55[a]				100

[a] Onset of morphologically transformed appearance.

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[3] H. F. CLARK, F. JENSEN and V. DEFENDI, Int. J. Cancer *9*, 599 (1972).
[4] F. MICHALSKI, M. M. COHEN and H. F. CLARK, Proc. Soc. exp. Biol. Med. *146*, 337 (1974).
[5] J. POPE and W. ROWE, J. exp. Med. *120*, 121 (1964).
[6] J. PONTEN, *Virology Monograph* (Springer-Verlag, New York 1971), vol. 8.

Table II. Transplantation of SV40 transformed gecko cells into autogeneic and allogeneic hosts

Animal no.	Cell line inoc.	No. transformed cells inoc. ($\times 10^7$)	Period transformed in vitro before inoc.	Route of inoc.	No. nontransformed cells inoc. ($\times 10^7$)	Route of inoc.	Results
			Autografts				
1	T1	5.9	20 passages in 143 days	s.c.[a]	1.0	s.c.	No tumor after 24 months
4	T4	1.3	17 passages in 126 days	s.c.	1.0	s.c.	No tumor at death after 13 months
5	T5	3.0	16 passages in 115 days	i.p.[b]	0		No tumor after 29 months
8	T8	2.1	11 passages in 83 days	s.c.	2.1	s.c.	No tumor after 29 months
			Allografts				
2	GE-1	0.03	9 passages in 95 days	s.c.	0.02	s.c.	No tumor at death after 11 months
3	GE-1	0.04	9 passages in 95 days	s.c.	0		No tumor after 36 months
7	GE-1	7.9	39 passages in 311 days	i.p.	0		No tumor at death after 8 months

[a] Subcutaneous; [b] Intraperitoneal.

antigen by the indirect immunofluorescence method. Morphological transformation took place in about 7–11 weeks (1–6) passages after infection at 30 °C, and was accompanied by a rise in the percentage of T antigen positive cells (Table I). The critera used to evaluate transformation were 1. change in morphology from fibroblast-like to epithelial-like, 2. change in growth pattern to a much higher saturation density, and 3. a rise in the percentage of T antigen positive cells. SV40 virus could not be detected in the culture medium after transformation had occurred.

Four animals were inoculated with their own SV40 transformed tail cells, and of these 3 were simultaneously inoculated in the opposite leg with non transformed cells of the same passage level as a control. (Table II). 3 animals were inoculated with the SV40 transformed gecko cell line of embryonic origin, GE-1, and of these one was simultaneously inoculated in the opposite leg with non transformed cells at the same passage level as a control. After 36 months 3 animals have died due to nutritional deficiencies, but no tumors were observed.

Discussion. Cell lines were easily started from the tails of geckos and transformed by SV40 virus, but tumors were not induced in geckos by either autografts or allografts of SV40 transformed cells. These results agree with the general conclusion reached by PONTEN[6] that SV40 transformed cells usually have a weak tumorigenic capacity after animal implantation, the exception being hamster cells. It has been shown in man[7], calves[8], rats[9], and mice[10] that autografts of SV40 transformed cells caused either no visible growth in the host or only a small nodule that invariably regressed. One explanation given for these results is that rejection may be due to the altered antigenicity of SV40 transformed cells. In a previous study of SV40 transformed gecko cells[3], evidence was found for a SV40 tumor specific transplantation antigen. This may explain why tumor growth was not achieved in these experiments. Also since these animals represent the highest group of vertebrates retaining a considerable measure of regenerative ability, neoplastic growth may have been controlled by regenerative power, as the work of Seilern-Aspang and Kratochwil[11] suggest is possible. Finally the number of cell inoculated may have been less than the critical minimum needed for tumor growth, or the cells may not have been passaged long enough at the time of inoculation to have become capable of unlimited growth. A definitive explanation must await further experimentation.

[7] F. JENSEN, H. KOPROWSKI, J. S. PAGANO, J. PONTEN and R. G. RAVDIN, J. natn. Cancer Inst. *32*, 917 (1964).
[8] H. DIDERHOLM and T. WESSLEN, Arch. ges. Virusforsch. *17*, 339 (1965).
[9] H. DIDERHOLM, R. BERG and T. WESSLEN, Int. J. Cancer *1*, 139 (1966).
[10] P. H. BLACK and W. P. ROWE, Proc. Soc. exp. Biol. Med. *114*, 721 (1963).
[11] F. SEILERN-ASPANG and K. KRATOCHWIL, *Regeneration in Animals and Related Problems* (Eds. N. KIORTSIS and H. TRAMPUSCH; North Holland Publishing, Amsterdam 1965), p. 452.

Ecdysterone-Induced Mortality and Inhibition of Feeding in Diapausing *Rhodnius prolixus* Stal

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Summary. Administration of ecdysterone was fatal to diapausing *Rhodnius prolixus;* the male adults and 19-week-old male fifth instar larvae were more susceptible than the 3-week-old larvae. The hormone also inhibited the feeding activity of the bugs.

Like other diapausing insects, growth and moulting in the diapausing *Rhodnius prolixus* is also initiated by ecdysone produced after blood meal[1]; the general physiology of the dormant bugs is also similar to that of other diapausing insects[2,3]. Administration of synthetic ecdysone has terminated diapause in some species of insects[4,5] suggesting the possibility of similar effects on *R. prolixus*. The present paper deals with the actions of synthetic ecdysterone on the survival and feeding behaviour of larval and adult *R. prolixus*.

[1] V. B. WIGGLESWORTH, Q., Jl. microsc. Sci. *77*, 191 (1934).
[2] V. B. WIGGLESWORTH, J. exp. Biol. *40*, 231 (1963).
[3] A. MANSINGH, Can. Ent. *103*, 987 (1971).
[4] C. M. WILLIAMS, Biol. Bull. *134*, 344 (1968).
[5] C. M. E. CLAY and C. E. VERNARD, Ann. ent. Soc. Am. *64*, 968 (1971).