480 nm, though the maximum of the fluorescence of the paraganglia was shifted to 495 nm (figure 3). For control purposes, formaldehyde-HCl-induced fluorescence of tryptophan in the tissue proteins was measured from the zymogen granules of the pancreatic acinar cells known to contrain chymotrypsinogen and trypsinogen with a relatively high number of tryptophane residues, which exhibit a bright fluorescence with this method[19, 21].The emission maximum of the fluorescence was at 495 nm, and the shape of the curve was similar to that recorded from the paraganglia of man (figure 3). After HCl treatment, intensely fluorescent granules could be observed in the cytoplasm of the PG-cells against the homogenous background fluorescence.

The 3fold increase in the formaldehyde-induced fluorescence of the paraganglia of man after acidification strongly suggests that there is a protein containing a relatively high number of tryptophane residues in the cytoplasmic granules. The molecular basis of the acid-catalyzed formaldehyde-induced fluorescence of tryptamines including tryptophane has been suggested to be the promotion of the formation of strongly fluorescent dihydro-β-carboline compounds[19]. Various modifications of the acid-catalyzed formaldehyde condensation reaction have been used for the localization of tryptophane-containing peptide hormones in the endocrine cells and of enzymes stored in the cytoplasmic granules in the exocrine cells[13–18]. Further evidence for the presence of tryptophane-containing protein was gained with the studies concerning the emission spectra, which can be used for the differentiation of the formaldehyde-induced fluorescence of various biogenic monoamines and amino acids at the cellular level[19]. The amino acid composition of the major protein constituents of the chromaffin granules of the adrenal medulla of several species is known and tryptophane has been found to be missing from these proteins[22]. Our observations suggest that there is a hitherto unknown protein in the granules of the paraganglionic cells of adult man. Considering the general characteristics of APUD-cells the results suggest, that the PG of man, which are widely distributed and well vascularized clusters of cells, do produce and store both catecholamines and tryptophane containing protein possibly of an endocrine nature.

17 A. Björklund, R. Håkanson and B. Falck, Acta physiol. scand., Suppl. 318 (1968).
18 S. Partanen, L. Rechardt and N. Bäck, Histochemistry *41*, 119 (1974).
19 L.-I. Larsson, R. Håkanson, N.-O. Sjöberg and F. Sundler, Gastroenterology *68*, 1152 (1975).
20 L.-I. Larsson, Commun. Dep Anat, Lund 3 (1975).
21 S. Partanen, Histochemistry *47*, 339 (1976).
22 H. Winkler, Neuroscience *1*, 65 (1976).

Serum activity inhibiting specific simian virus 40-induced transplantation resistance and its correlation with primary SV40 tumors appearance in hamsters

E. A. Volpe[1]

Laboratory of Tumor Immunology, Cancer Research Center, USSR Academy of Medical Sciences, Kashirskoye shosse 6, Moscow 115 478 (USSR), 8 October 1976

Summary. Using the modified technique of transplantation test, ITR serum activity was found in most (14 out of 21) individual hamster sera obtained during the latent period of primary SV40 carcinogenesis (60 days after virus infection when newborn). On the other hand, as a rule, no ITR activity was observed in the sera of the same hamsters after tumor appearance and during their growth. ITR activity rapidly disappeared from sera of hamsters neonatally infected with SV40 after their successful immunization with the same virus during the latent period. There appears to be a correlation between the presence of ITR serum factor during the latent period and the subsequent primary SV40 tumor appearance in hamsters.

The factor(s) blocking lymphocytes cytotoxic activity in sera of individuals bearing primary and transplantable tumors have been demonstrated mainly with in vitro assays[1–8]. The interest of these factors, however, is connected with their supposed and unfavourable role for the organism in vivo. The studies of the role of humoral factors, and especially blocking serum activity in vitro and in vivo in hamsters bearing SV40[9] induced tumors, are scanty and contradictory[10–15]. Therefore, the present in vivo studies were carried out in order to investigate possible influence on tumor cells of the sera samples obtained from individual Syrian hamsters during different periods of primary SV40-induced carcinogenesis. For this purpose, the sera samples, as well as control NHS, were used for the pretreatment of SV40 test-tumor cells in vitro and subsequent challenge of such pretreated cells in immune (SV40 virus-inoculated) and normal adult hamsters.

Materials and methods. Syrian hamsters of both sexes were used. Their origin has been described[16]. SV40 virus, strain No. 128, with a titer of $10^{7.4}$–$10^{7.5}$ $TCID_{50}$/1.0 ml, was used for infection of newborn hamsters and for immunization of adult animals. Newborn hamsters not older than 18 h were infected S.C. with 0.2 ml of the undiluted SV40 virus. Some of the animals neonatally infected with SV40 were immunized by reinfection with the same virus (in a volume of 0.5 ml) i.p. during the latent period (60 days after birth). Such immunization can effectively prevent tumor appearance in hamsters inoculated by SV40[17–20]. As transplantable test-tumor throughout these studies hamsters SV40-induced sarcoma was used. From this tumor, an in vitro tissue culture cell line designated as E-1 was established. The origin and tumorigenicity of the E-1 cell line have been reported[16, 21]. In these studies E-1 cells from the 6th to the 100th in vitro passages were used. Sera from each hamster neonatally infected with SV40 were obtained repeatedly at the different stages of primary SV40 carcinogenesis. Control sera were obtained from untreated hamsters of the same age. All sera were prepared by the retroorbital sinus or cardiac puncture, heat-inactivated at 56 °C for 30 min and stored at –20 °C until use. For pretreatment of the E-1 test-tumor cells with hamster sera in vitro 0.05 ml of undiluted serum was added to the known number of serially 10-fold-diluted E-1 cells. The mixtures of the E-1 cells pretreated either with sera tested or with NHS were incubated at 37 °C for 45 min and challenged both in 4–5 immunized and 4–5 normal adult hamsters s.c. Modified technique of transplantation test was used. Details of the technique

Table 1. Characteristics of Syrian hamsters studied

Animals group	Neonatally infected with SV40[a]	Immunized with SV40 during the latent period[b]	Tumor incidence[c] Total	%	Mean duration of the latent period (days ± SD)
1	No	No	0/25	0	
2	Yes	No	25/50	50	185 ± 68
3	Yes	Yes	3/23[d]	13	316 ± 199[e]

[a, b] See text. [c] Number of hamsters developing tumors/No. of hamsters infected. [d] The frequency of tumor incidence of group 3 was significantly lower than those of non-immunized of group 2 by χ^2-test ($p < 0.01$). [e] Mean tumor latent period of group 3 was significantly longer than that of group 2 by Student's t-test ($p < 0.01$).

used have been given previously[16, 21]. The method of Reed and Muench[22] wat used to calculate the logarithm of TrD_{50} in immune and normal animals. In all experiments, a RI was calculated. Serum tested was evaluated as possessing ITR activity if it significantly reduced the log TrD_{50} of pretreated test-tumor cells in immune animals (as compared to control ones). In this case, the decrease of RI was considered as connected predominantly with the protection of test-tumor cells by serum factor in immune animals. However, the same decrease of RI could be observed in some cases connected with increase of the log TrD_{50} of serum pretreated E-1 cells in normal hamster. In the latter case, serum activity was considered as toxic and possibly non-specific.

Results and discussion. Characteristics of Syrian hamsters used as a source of sera tested are described in table 1. Group 1 received no treatment and served as controls. 2 independent series of experiments were carried out.

1st series. The effect of hamster sera on transplantation resistance was studied. Individual sera from 24 hamsters neonatally infected with SV40 were tested. In 12 of these hamsters, tumors appeared (group 2A) and sera from the animals were obtained at the latent period (60 days after birth), after its termination on the day of the primary tumors appearance, and later twice on 19–36th and 45–57th day of progressive tumor growth. Sera from other 12 SV40-infected hamsters in which no tumor developed (group 2B) were obtained on day 60 after infection with SV40.

The results summarized in table 2 show that 7 out of 12 sera obtained during the latent period from hamsters of group 2A were capable of protecting test-tumor cells in immune hamsters ($p < 0.01$), in contrast to NHS or sera from the animals of group 2B. As a rule, no significant ITR activity in the sera of the same hamsters after tumor appearance and during their growth was observed. Only 2 out of 35 (6%) serum samples tested after tumor appearance inhibited transplantation resistance after pretreatment of the E-1 cells. In both cases, the sera were obtained from hamsters in which earlier (during the latent period) ITR serum activity was also present.

2nd series. The effect of the immunization with SV40 during the latent period on the frequency of tumor appearance and possibility of detection of ITR serum factor was studied. Some of the neonatally SV40-infected hamsters were repeatedly inoculated with the same virus 60 days later. The immunization was successful and prevented tumor growth in most hamsters infected with SV40 when newborn. The frequency of tumor appearance in immunized animals (group 3) was significantly lower than those of non-immunized group 2 ($p < 0.01$). Furthermore, the immunization led to a significant prolongation of the mean tumor latent period of group 3 (table 1).

Individual sera from 9 hamsters neonatally infected with SV40 and immunized during the latent period were tested for their capacity to inhibit transplantation resistance. Sera from 6 of these animals in which no tumor developed (group 3A) were obtained twice during the latent period: before the reinfection with SV40 (60 days after birth), and 21 days after this immunizing procedure (81 days after birth). In those 3 animals in

1 Acknowledgment. The author wishes to thank Dr Galina I. Deichman for continuing advice and encouragement during the course of these studies.

2 K. E. Hellström and I. Hellström, in: Advances in Immunology, vol. 18, p. 209. Ed. F. J. Dixon and H. G. Kunkel. Academic Press, New York 1974.
3 G. H. Heppner, in: Immunological Aspects of Neoplasia. Series Haematologica, vol. 5, p. 41. Ed. K. G. Jensen and S. A. Killmann. Munksgaard, Copenhagen 1972.
4 H. O. Sjögren, in: Methods in Cancer Research, vol. 10, p. 19. Ed. H. Busch. Academic Press, New York 1973.
5 R. W. Baldwin, M. J. Embleton, M. R. Price and R. A. Robins, Cancer *34*, Suppl. *1*, 1452 (1974).
6 G. Steele, Jr, H. O. Sjögren, J. E. Rosengren, C. Lindström, A. Larsson and L. Leandoer, J. nat. Cancer Inst. *54*, 959 (1975).
7 R. W. Baldwin and R. A. Robins, Br. Med. Bull. *32*, 118 (1976).
8 I. Hellström and K. E. Hellström, in: In vitro Methods in Cell-Mediated and Tumor Immunity, p. 533. Ed. B. R. Bloom and J. R. David. Academic Press, New York 1976.
9 The abbreviations used are:
SV40, simian vacuolating virus 40;
NHS, normal hamster sera;
$TCID_{50}$, median tissue-culture infective dose;
ITR, serum activity inhibiting transplantation resistance;
TrD_{50}, dose of tumor cells causing growth of transplanted tumors in 50% of the animals;
RI, a resistance index;
L_{60}, sera obtained from hamsters SV40-infected when newborn during the latent period 60 days after birth;
L_{81}, sera obtained from hamsters 21 days after immunization with SV40 during the latent period (81 days after birth);
T_0, sera obtained from the hamsters at the day of appearance of palpable primary tumors;
T_{18-36} and T_{45-57}, sera obtained from the hamsters on 18–36th and 45–57th days of the primary tumors growth respectively;
SD, standard deviation.
10 J. H. Coggin, Jr, K. R. Ambrose, P. J. Dierlam and N. G. Anderson, Cancer Res. *34*, 2092 (1974).
11 M. H. Goldrosen and P. B. Dent, Fed. Proc. *34*, 1041 (1975), Abstract.
12 M. H. Goldrosen and P. B. Dent, Br. J. Cancer *32*, 667 (1975).
13 J. W. Blasecki, Fed. Proc. *35*, 226 (1976), Abstract.
14 S. O. Prather and R. N. Lausch, Int. J. Cancer *17*, 380 (1976).
15 S. O. Prather and R. N. Lausch, Int. J. Cancer *18*, 820 (1976).
16 E. A. Volpe, Neoplasma, Bratisl. *23*, 345 (1976).
17 G. I. Deichman and T. E. Kluchareva, Nature, Lond. *202*, 1126 (1964).
18 G. I. Deichman and T. E. Kluchareva, Virology *24*, 131 (1964).
19 G. I. Deichman and T. E. Kluchareva, J. nat. Cancer Inst. *36*, 647 (1966).
20 G. I. Deichman, in: Advances in Cancer Research, vol. 12, p. 101. Ed. G. Klein and S. Weinhouse. Academic Press, New York 1969.
21 E. A. Volpe, Vest. Akad. med. Nauk SSSR, No. 2, 26 (1976).
22 L. J. Reed and H. Muench, Am. J. Hyg. *27*, 493 (1938).

which such immunization was unsuccessful and tumors appeared (group 3), sera were obtained at the day of primary tumor appearence and later on day 18–20 and 48 of progressive tumor growth.

5 out of 6 sera obtained from hamsters of group 3A and 2 out of 3 sera from group 3B exhibited ITR activity before the immunization ($p < 0.5$ and $p < 0.01$, respectively). The immunization resulted in a rapid disappearance of ITR serum activity from the animals of group 3A. However, sera obtained from 3 hamsters of group 3B during the latent period 3 weeks after the immunization possessed ITR activity. Only 6 serum samples obtained from these animals after tumor appearance were available. One of these samples possessed ITR activity (T_0), and some were found to be cytotoxic. None of the control NHS tested in both series of experiments had detectable ITR activity (table 2).

The results indicate that ITR serum factor is present in most (14 out of 21; 67%) individual hamster sera obtained during the latent period of the primary SV40 carcinogenesis, whereas no such activity, as a rule, could be detected in the animals after tumor appearance and during their growth. The correlation between the presence of ITR serum factor in hamsters during the latent period of SV40-induced carcinogenesis and the subsequent primary tumor appearance may be suggested, since: a) ITR factor was found in most animals at an early stages of tumor development; b) no ITR activity appeared in animals in which no tumor developed, and c) the factor disappeared or was retained in the animals, depending on the effectiveness of prophylactic immunization against tumor with SV40 (table 3).

It seems to indicate that ITR serum factor found to be present in hamsters during the primary SV40-induced carcinogenesis observed in our in vivo studies differs from serum lymphocyte blocking factor revealed by others with in vitro assays not only by its dynamics, but also by its nature. It is unclear whether there is any connection between cytostatic (C) antibodies described by Ambrose and Co-workers[23] in 3-week-old hamsters SV40 virus infected when newborn, and ITR serum factor observed in hamsters during the latent

23 K. R. Ambrose, N. G. Anderson and J. H. Coggin, Jr, Nature, Lond. *233*, 321 (1971).

Table 2. Effect of hamster sera obtained at different stages of primary SV40 viral carcinogenesis on SV40 virus-induced transplantation resistance

Animal group	Tumor incidence in sera donors[a]	Sera used for tumor cells pretreatment in vitro	Results of transplantation tests with pretreated E-1 test-tumor cells[b] log TrD_{50} in Immune[c] hamsters	Normal hamsters	log RI[d]		Frequency of sera with ITR activity[e]
1st series							
1	0/12	NHS (control)	3.9 ± 1.06	2.5 ± 1.09	1.4 ± 0.27	(12)	0/12
2A	12/12	L_{60}	2.9 ± 0.48[i]	2.2 ± 0.50	0.7 ± 0.45[j]	(5)	7/12
		T_0	3.4 ± 0.37	1.8 ± 0.25[g]	1.6 ± 0.41	(5)	1/12
		T_{19-36}	3.3 ± 0.37	1.9 ± 0.61	1.4 ± 0.45	(4)	0/11
		T_{45-57}	3.9 ± 1.00	2.7 ± 1.13	1.2 ± 0.47	(8)	1/12
1	0/2	NHS (control)	4.0 ± 0.07	2.8 ± 0.00	1.2 ± 0.07	(2)	0/2
2B	0/12	L_{60}	4.1 ± 0.13	3.0 ± 0.21	1.1 ± 0.18	(2)	0/12
2nd series							
1	0/4	NHS (control)	3.5 ± 0.34	2.1 ± 0.15	1.4 ± 0.22	(4)	0/4
3A[f]	0/6	L_{60}	2.7 ± 0.52[g]	1.9 ± 0.21	0.8 ± 0.33[h]	(2)	5/6
		L_{81}	3.4 ± 0.52	2.2 ± 0.71	1.2 ± 0.36	(3)	0/6
1	0/7	NHS (control)	3.6 ± 0.29	2.1 ± 0.17	1.5 ± 0.33	(7)	0/7
3B[f]	3/3	L_{60}	2.9 ± 0.10[i]	1.7 ± 0.45	1.2 ± 0.35	(3)	2/3
		L_{81}	3.0 ± 0.44[g]	2.6 ± 0.40[h]	0.4 ± 0.21[j]	(3)	3/3
		T_0	3.2 ± 0.53	2.0 ± 0.26	1.2 ± 0.76	(3)	1/3
		T_{18-20}	3.6 ± 0.21	2.7 ± 0.07[i]	0.9 ± 0.28	(2)	0/2
		T_{48}	4.4	3.7	0.7	(1)	0/1

[a] Number of hamsters developing tumors/No. of hamsters tested. [b] Results are presented as mean ± SD. The significance was determined with Student's t-test. Mean ± SD was calculated on total number of specimens of hamster sera for each category represented. Number in parentheses indicates the number of independent experiments. [c] Inoculated with SV40 virus 5–23 days before challenge. [d] RI, the ratio of TrD_{50} (the logarithm of the cell dose causing growth of transplanted tumors in 50% of the challenged animals) in resistant (immune) animals to TrD_{50} in normal ones. [e] Number of hamsters sera possessing ITR activity/No. of individual sera tested. [f] Immunized with SV40 during the latent period (60 days after birth). [g] $p < 0.05$; [h] $p < 0.02$; [i] $p < 0.01$; [j] $p < 0.001$. Values without marks indicate no significant difference vs controls ($p > 0.05$).

Table 3. Correlation of ITR serum activity with primary SV40 tumors appearance in hamsters

Animal group	Immunization with SV40 during the latent period (60 days after birth)	Tumor appearance	Frequency of sera with ITR activity during different stages of SV40 carcinogenesis				
			L_{60}	L_{81}	T_0	T_{18-36}	T_{45-57}
2A	No	Yes	7/12 (58%)	NT[a]	1/12 (8%)	0/11	1/12 (8%)
2B	No	No	0/12	NT[a]			
3A	Yes	No	5/6 (83%)	0/6			
3B	Yes	Yes	2/3 (67%)	3/3 (100%)	1/3 (33%)	0/2	0/1

[a] Not tested.

period of SV40 carcinogenesis in our experiments. The role of ITR serum factor in vivo is still unknown. The appearance of ITR factor in sera of hamsters neonatally infected with SV40 may possibly reflect the process of primary carcinogenesis and may be favourable for tumor development. The specificity and nature of ITR serum factor remains to be examined. Apparantly, detection of ITR humoral factor at an early stage of carcinogenesis may provide a useful tool not only for a better understanding of the immunological aspects in viral-induced tumorigenesis, but also it may be of diagnostic and prognostic value.

On the turnover of exogenous ferritin in the cephalopod optic gland. A microprobe study[1]

D. Froesch, Katharina Mangold[2] and W. Fritz

Sektion Elektronenmikroskopie, Universität Ulm, D–79 Ulm (Federal Republic of Germany), 25 May 1977

Summary. X-ray energy emission spectra of iron show that horse spleen ferritin, after injection into the blood of the octopus, is taken up, accumulated and disposed of again by lysosomes of the optic gland.

Electron microscopic evidence has been produced for the uptake of ferritin by the stellate (or main or chief) cells of the cephalopod optic gland[3], a presumed endocrine organ[4]. In the present note, we demonstrate by the use of energy dispersive X-ray analysis, that exogenous ferritin is accumulated transiently in lysosomes of the stellate cells with respect to its concentration in the blood.

8 octopuses (*Octopus vulgaris*), anaesthetized with 2% ethanol in seawater, were injected into the aorta with 1 g/kg of horse spleen ferritin (Fluka, twice cristallized, cadmium-free) and killed at intervals of 15 min, 1 h, 4 h and 24 h. The optic glands, pin head-sized organs, situated upon the optic tracts, were fixed in 2% osmic acid (in a cacodylate-buffered Ringer of 1180 mosm) and processed for conventional electron microscopy. Unstained sections of approximately 1000 Å thickness, mounted upon plastic grids, were examined in a LINK EDX 290 microprobe analyser, attached to a Zeiss EM 10.

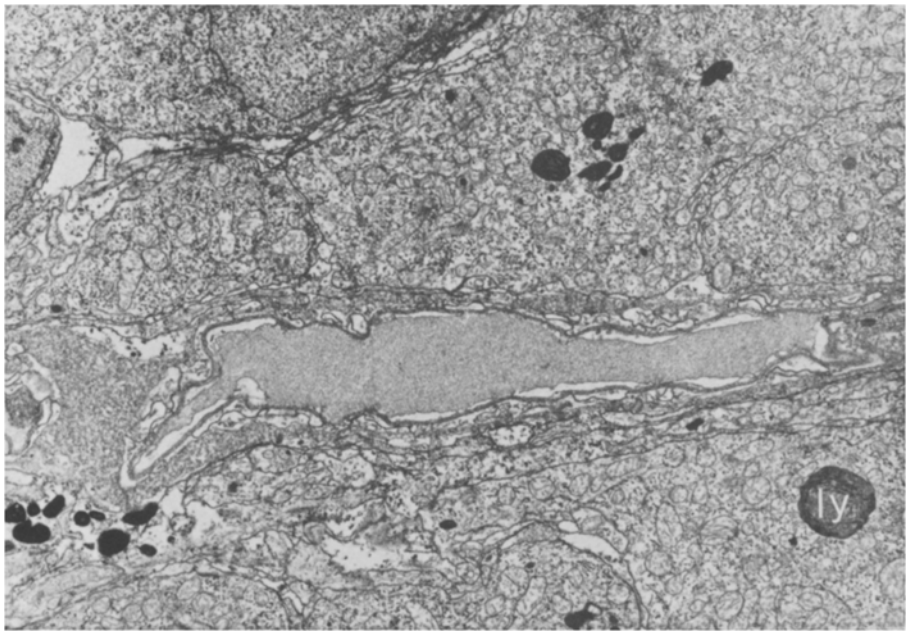

Fig. 1. Survey electron micrograph of an optic gland capillary of an octopus, injected with 1 g/kg of ferritin 1 h before fixation. This protein is taken up and disposed of again by the sort of lysosomes (ly) which are shown in the adjacent stellate cells. ×5460.

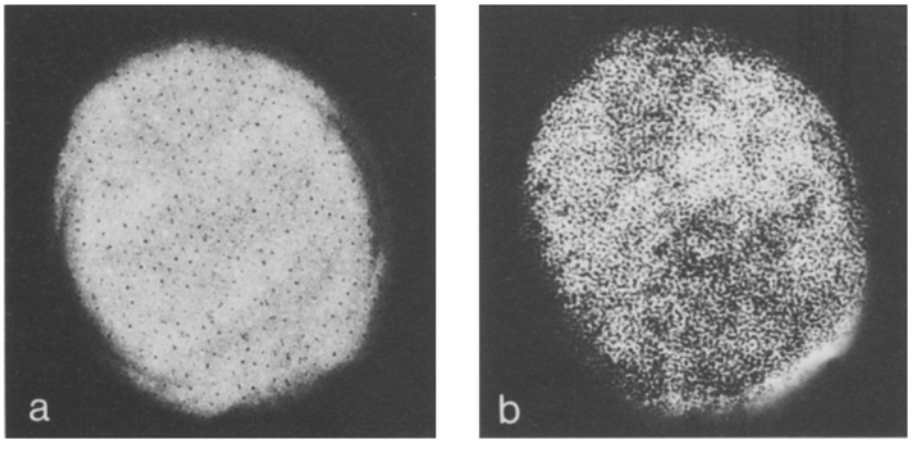

Fig. 2. Electron micrographs of (*a*) a blood vessel and (*b*) an optic gland lysosome, taken during the X-ray analysis. The blood vessel was fixed 1 h and the lysosome 4 h after the injection of ferritin. The ferritin molecules form a mixed crystal with the hemocyanin molecules in the blood of the octopus. In both micrographs, the diameter of the beam is 0.7 μm.