represents a denatured lipoprotein, the possibility of its precipitation *in vivo* must be considered. Such denatured proteins might alter vascular permeability as well as all membrane permeability.

With the exception of the persistence of the beta lipoprotein positioned curve in the experimental sera, which presumably accounts for the increase in the beta globulin fraction previously reported in this condition, the remainder of the immunophoretic pattern remains like that found in normal sera. The previously observed decrease in albumin in chronic E-deficient chicks is not reflected in these tests, which are qualitative, not quantitative. The unusual appearance of the 7S gamma globulin curve in some experimental preparations is not explained.

Clinical findings of retarded growth, feather development and other skin changes are expression of the lack of vit E in the diet. This vitamin has a definitive growth promotion factor. In addition, it facilitates the use of vit A which, even though present to excess in the diet, is apparently insufficiently utilized, as evidenced by the skin changes.

Summary and conclusions. 1. Eleven one-day-old chickens were placed on diets deficient in vitamin E or any synthetic antioxidant and on sulfur amino acids. Eight chickens of the same hatch were kept on normal stock diets for comparison. All of the experimental chickens developed paralyses of breast muscles, and weakness of leg muscles in from 6 to 8 weeks. Normal chickens were free from symptoms. Both sets of chickens were sacrificed at the same time. Blood sera, obtained by cardiac puncture, were examined by immunophoresis, using anti-chicken serum antibodies in rabbit serum. 2. Significant immunophoretic finding was the persistence of a precipitation curve in the beta lipoprotein mobility range in sera of experimental chickens, which is normally not found in chickens of the same age. The remainder of the immunophoretic preparations pattern was alike in experimental and normal sera. 3. Interpretation of the altered serum protein in chronic vit E-deficient chickens is discussed in terms of altered lipid metabolism.

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Stimulation of DNA Synthesis in Human and Hamster Cells by Human Adenovirus Types 12 and 5.* (31241)

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Human adenovirus type 12 was found to be oncogenic for newborn hamsters(1), and transformation of hamster cells was reported following exposure to this virus *in vitro*(2,3). Transformation *in vitro* has been much more difficult to obtain with adeno-12 virus than with polyoma virus and SV40 virus. The nature of the association between polyoma virus and hamster cells has been studied by several authors. Hamster fibroblasts, including the stable line BHK 21, show little or no degeneration, but a proportion of the cells undergo neoplastic transformation(4). Fraser and Gharpure(5) have shown by immunofluorescence that virus antigens persist in the BHK

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21 cells through several divisions. Bourgaux (6), using radioactive polyoma virus, reported that most of the radioactivity remaining acid insoluble was lost from the cytoplasm by rejection into the medium and by association with the nuclear fraction and that the nuclear associated radioactivity remained constant for 4 to 6 days. It was previously reported that in hamster cells infected with adeno-12 virus, production of neither mature virus(7) nor virion structural antigen(8) took place. The present study was undertaken to investigate the interaction between hamster cells and adeno-12 virus using radioautography of thymidine-H^3 (TD_r-H^3). Adeno-5 virus, which does replicate in hamster cells and has been presumed nontumorigenic, was also employed as an aid to clarify the action of adeno-12 virus.

Materials and methods. Virus. Prototype adeno-5 and adeno-12 viruses passaged in human KB cells in this laboratory were used. Stock virus was prepared by homogenizing infected KB cells in Eagle's medium, and by centrifugation to clarify. The titers of stock virus were $10^{8.0}$ PFU per ml in KB cells for adeno-5 virus, and $10^{7.5}$ $TCID_{50}$ per ml in human embryonic kidney cells for adeno-12 virus. For all experiments, virus suspension was treated with an equal volume of crystalline trypsin in phosphate buffered saline (PBS) (0.25%) for 30 minutes at 37°C to inactivate soluble toxin. Trypsin action was stopped by an equal amount of soybean trypsin inhibitor (0.25% in PBS solution).

Primary Syrian hamster cell cultures. "Whole" embryonic cell cultures were prepared by mincing and trypsinization of embryos from which the head and legs were first removed. "Newborn" kidney cell cultures were prepared by mincing and trypsinization of kidneys removed from 24- to 72-hour-old hamsters. In either case, trypsinized cells were dispensed into petri dishes (60 mm) containing coverslips.

All cultures were grown and maintained in Eagle's medium supplemented with 10% calf serum. After infection calf serum was replaced by 5% agammaglobulin calf serum.

Experimental procedure. Two sets of cell cultures were prepared in each series of experiments, one for radioautographic study and another for hematoxylin-eosin staining. After a monolayer had formed, the medium was removed, 1 ml of virus suspension was added and the cells incubated for 2 hours at 37°C to permit virus adsorption. Then the inoculum was discarded, the cells washed twice with PBS and 5 ml of fresh medium were added. Input multiplicity was approximately 10 to 20 PFU/cell for adeno-5 infection and 3 to 7 $TCID_{50}$/cell for adeno-12 infection.

For radioautography, 5 ml of Eagle's medium containing H^3-thymidine (1.2 μc/ml) were introduced into each petri dish at the indicated times after discarding the old medium. The cultures were incubated at 37°C for 1 hour with agitation every 5 minutes. Then coverslips were rinsed 3 times with cold PBS and fixed with Bouin's solution. After washing with 70% ethanol, coverslips were air-dried and put in 0.2 N $HClO_4$ solution for 60 minutes at 4°C to remove acid-soluble fraction and washed thoroughly with water. Eastman Kodak NTB 2 emulsion was applied for radioautography according to the dipping method of Kato *et al*(9). Exposure time was usually 5 days in the refrigerator, with silica gel as a drying agent. After development the cells were stained with hematoxylin-eosin. Control uninfected cultures were subjected to the same procedure except for the addition of virus suspension.

Results. TD_r-H^3 uptake of infected KB cells. The growth of adeno-5(10) and adeno-12 viruses(7) in HeLa monolayer cells was investigated by Wilcox *et al* and Kitamura *et al.* In our experiments, the time course of growth of either virus in KB monolayer cells was essentially the same as that in HeLa cells, requiring 36 hours and 48 hours for one growth cycle of adeno-5 and adeno-12 virus, respectively. Two-day-old KB cell cultures were infected with either adeno-5 or adeno-12. At 14 to 15 hours post-infection (PI) 95 and 68% of the cells became labelled in adeno-5 and adeno-12 infection, respectively, while only 29% of uninfected control cells became labelled (Table I). However, analysis of distribution of grain numbers per labelled cell showed that while control cells

TABLE I. Thymidine-H^3 (TD_r-H^3) Uptake by Monolayer Cultures of Human KB Cells (Radioautography).

Hr post-infection before adding TD_r-H^3 for 1 hr	Uninfected control	Adeno-5 infected		Adeno-12 infected	
	% Cells* labelled	% Cells* labelled	% Cells* with inclusions	% Cells* labelled	% Cells* with inclusions
14	29.0	95.3	7	68.0	0
24	32.1	97.1	99	81.0	0
36	ND†	ND	ND	ND	72
48	ND	ND	ND	ND	89

* 1000 cells were examined in each sample. † Not done.

were for the most part heavily labelled, infected cells were rather lightly labelled (Fig. 1). The trend was the same at 24 to 25 hours PI. The percentage of cells with typical nuclear inclusions was 99 for adeno-5 at 24 to 25 hours PI, and 89 for adeno-12 at 48 to 49 hours PI indicating the high multiplicity of infection in this experiment. The location of incorporated TD_r-H^3 in the nuclei was closely associated with the viral inclusion bodies (Fig. 2, 3).

TD_r-H^3 uptake of infected hamster newborn kidney cells. The growth of adeno-5 virus in cultures of newborn hamster kidney and of hamster embryo cells is shown in Fig.

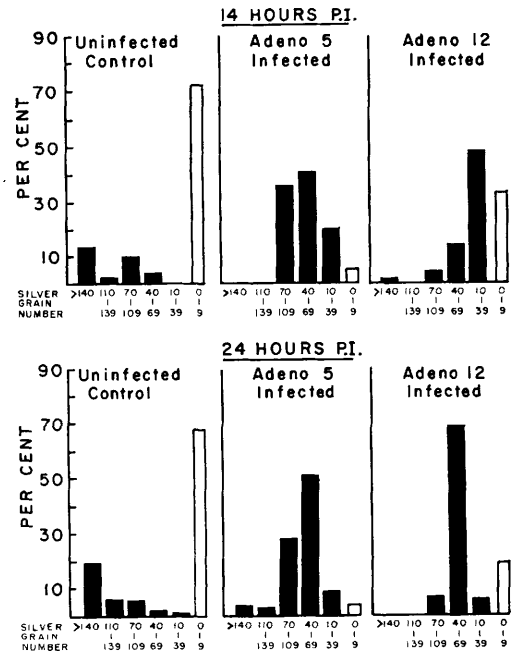

FIG. 1. Distribution of silver grain numbers in monolayer cultures of human KB cells (radioautography).

4. The growth cycle is not significantly different from that in KB cells. In contrast adeno-12 virus, which has a similar growth cycle in human cells, does not replicate infectious virus(7) in hamster cells. When 5-day-cultured newborn hamster kidney cells, composed predominantly of epithelial cells, were infected with adeno-5 or adeno-12 virus, both the percentage of labelled cells and the intensity of labelling increased significantly as compared with uninfected control cell cultures (Table II and Fig. 5).

The mitotic index decreased after infection with either virus. In adeno-5 infection, typical intranuclear viral inclusion bodies were found, mostly in epithelial cells, amounting to 90% of the total cells at 36 to 37 hours PI. The location of incorporated TD_r-H^3 in the nuclei was again closely associated with the inclusion bodies, just as in KB cells. No inclusion bodies were formed in the adeno-12-infected hamster kidney cultures.

TD_r-H^3 uptake of hamster embryonic cells. Four-day-old cultures of hamster embryo cells, composed predominantly of fibroblastic cells with a small fraction of epithelial cells, were infected with adeno-5 or adeno-12 virus. Characteristic intranuclear inclusion bodies of adeno-5 virus were found mostly in the epithelial cells, so that only 7.7% or less of the total cells formed inclusion bodies (Table III). This together with the data of Table II indicates that fibroblastic cells are far less susceptible to adeno-5 virus, and probably to adeno-12 virus, than are epithelial cells. The percentage of labelled cells increased only slightly following infection with either virus, as compared to hamster kidney cultures, probably due to lower susceptibility of the

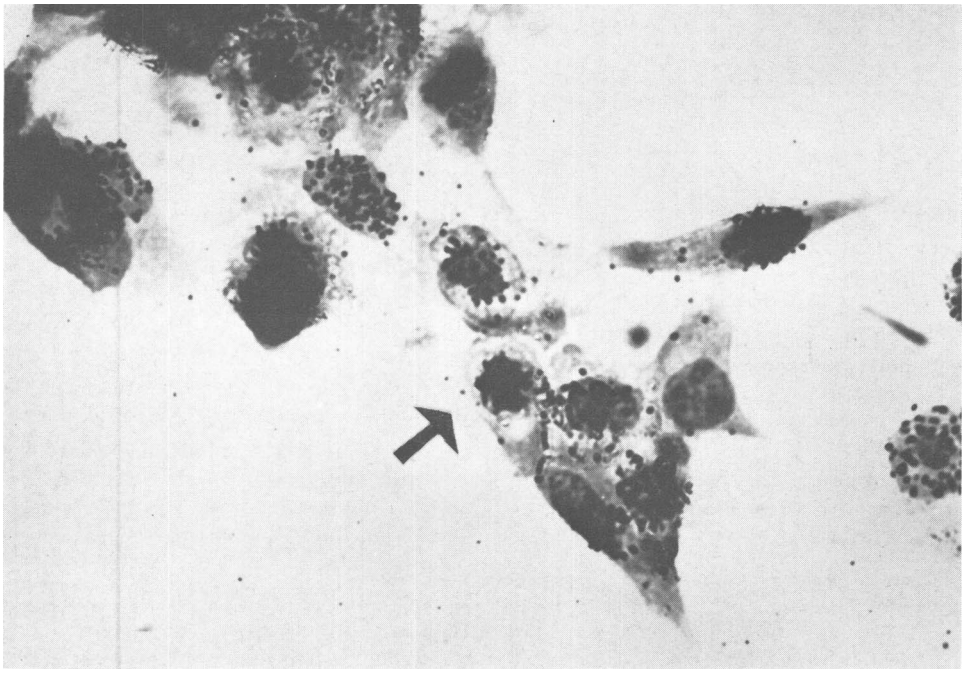

FIG. 2. Radioautogram of adeno-12 virus-infected human KB cells incubated for 1 hr with TD_r-H^3, at 48 hr post-infection. Labelled intranuclear inclusions were observed (arrow) (400 ×).

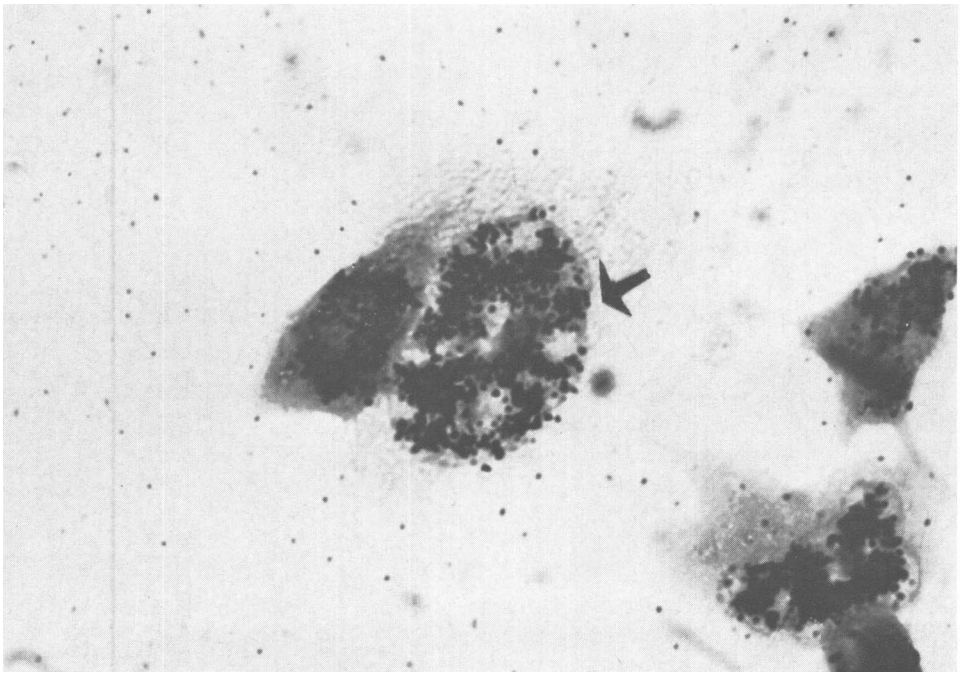

FIG. 3. Radioautogram of adeno-5 virus-infected human KB cells incubated for 1 hr with TD_r-H^3, at 24 hr post-infection. Intranuclear inclusions were observed densely labelled (arrow) (1000 ×).

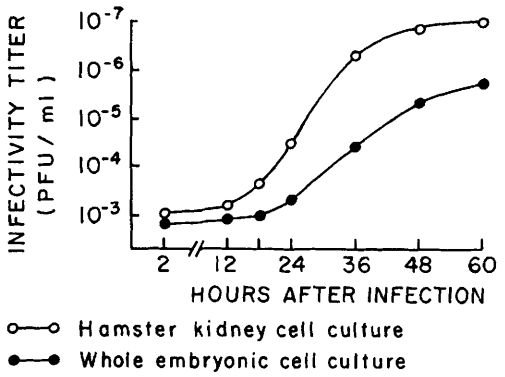

FIG. 4. Growth of adeno-5 virus in newborn hamster kidney cell culture and whole hamster embryo cell culture. One ml aliquots of adeno-5 virus suspension were inoculated into 2 oz bottle cultures of hamster kidney cells or whole embryo cells (input multiplicity 10-20). After adsorption time of 2 hr, cell sheet was washed 3 times with PBS and 5 ml of fresh medium added. At indicated time, cells were harvested, lysed by freezing and thawing 6 times and assayed for infectivity by plaque method in KB cells.

predominantly fibroblastic cells of the hamster embryo cultures.

Discussion. Despite much effort by many investigators, the mechanism of cell transformation by tumorigenic viruses has not been fully clarified. The present study revealed that, whereas production of neither mature virus nor virion structural antigen has been reported, approximately one-third of the hamster kidney cells are susceptible to adeno-12 virus infection, as indicated by increased DNA synthesis. This together with the facts that adeno-12 induces malignant transformation in hamster cells, and that the type specific, complement fixing adenovirus C antigen is produced in these tumor cells(11) indicates that adeno-12 is indeed infectious for hamster cells, but has an incomplete growth cycle therein. In the case of adeno-5 infection, most of the epithelial cells are susceptible to this virus, and the increased DNA synthesis is more striking.

In contrast to the susceptibility of the epithelial cells of either hamster kidney cultures or hamster embryo cultures, the predominantly fibroblastic cells of hamster embryo cultures were relatively less susceptible to the thymidine-H^3 uptake stimulating effect of adeno-5 and adeno-12, and to intranuclear inclusion formation by adeno-5. Whereas malignant transformation of newborn hamster cells by adeno-12 virus is much more difficult to achieve *in vitro* than *in vivo,* it may be significant that what success has been achieved *in vitro* has been largely with hamster kidney cells(2,3,12). In only one instance has a hamster embryo cell culture been reported to give transformation(12). The tumors formed by adeno-12 *in vivo* are usually undifferentiated and of undetermined histogenesis, although they have sometimes been referred to as sarcoma(1), and as having epithelioid characteristics(13), or as being lymphosarcoma resembling a reticulosarcoma(14). Tumors are invariably formed at any site of injection in the newborn hamster so that the cell of origin must be widespread (15). The only remote primary tumors have been in the liver(15). The present studies and *in vitro* transformation studies(12), suggest that an epithelioid cell may more likely be the cell of origin than a fibroblastic cell. A careful study of early histogenesis failed to reveal conclusively a cell type of origin but suggested possible mesothelial origin in some cases(16).

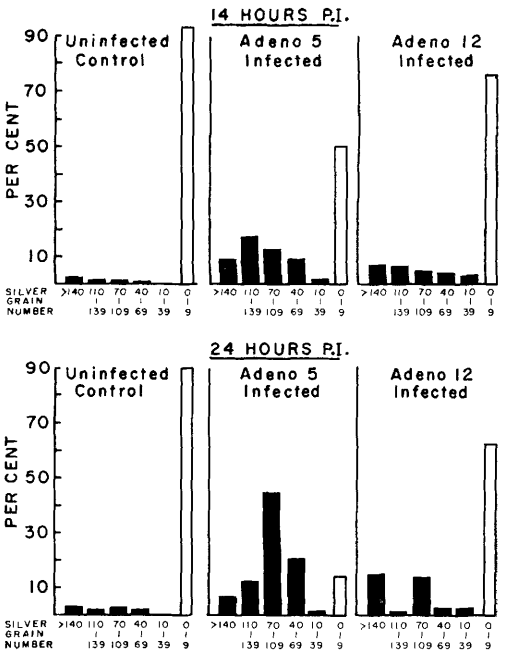

FIG. 5. Distribution of silver grain numbers in monolayer cultures of newborn hamster kidney cells (radioautography).

TABLE II. Thymidine-H^3 (TD_r-H^3) Uptake by Monolayer Cultures of Newborn Hamster Kidney Cells (Radioautography).

Hr post-infection before adding TD_r-H^3 for 1 hr	Uninfected controls		Adeno-5 infected			Adeno-12 infected		
	% Cells* labelled	Mitotic* index	% Cells* labelled	Mitotic* index	% Cells* with inclusions	% Cells* labelled	Mitotic* index	% Cells* with inclusions
14	7.0	.5	49.9	0	0	24.8	0	0
24	9.7	.5	84.8	.2	31	37.0	.3	0
36	12.4	.4	88.0	0	90.4	25.2	0	0
48	7.0	ND†	60.2	ND	ND	25.2	ND	0
60	1.3	ND	50.0	ND	ND	10.9	ND	0

* 1000 cells were examined in each sample. † Not done.

TABLE III. Thymidine-H^3 (TD_r-H^3) Uptake by Monolayer Cultures of Hamster Embryo Cells (Radioautography).

Hr post-infection before adding TD_r-H^3 for 1 hr	Uninfected control	Adeno-5 infected		Adeno-12 infected	
	% Cells* labelled	% Cells* labelled	% Cells* with inclusions	% Cells* labelled	% Cells* with inclusions
14	31.2	42.6	0	44.7	0
18	22.4	28.7	0	31.1	0
24	17.0	25.5	5.8	26.3	0
36	10.0	29.3	7.7	10.9	0
48	7.0	28.6	5.5	9.3	0

* 1000 cells were examined in each sample.

The present study does not permit a definitive conclusion as to whether the enhanced incorporation of TD_r-H^3 in either adeno-5 or adeno-12 infection was caused by viral DNA synthesis or by chromosomal (cellular) DNA synthesis. However, the facts that the mitotic index decreased after either adeno-5 or adeno-12 infection and that incorporation sites of TD_r-H^3 were closely correlated with viral intranuclear inclusion bodies in adeno-5 infection, which inclusions are reported to contain viral antigen(17), suggest that the increased incorporation was the result of viral DNA synthesis.

Summary. The effect of adeno-12 and adeno-5 viruses on the incorporation of thymidine-H^3 by cultures of human KB cells, newborn hamster kidney cells, and hamster embryo cells was investigated by radioautography. *In KB cultures,* infection with either adeno-5 or adeno-12 virus significantly increased the percentage of labelled cells, but the number of silver grains per labelled cell was somewhat lower than for control cultures. *In hamster kidney cells,* infection with either adeno-5 or adeno-12 decreased the mitotic index but significantly increased both the percentage of labelled cells and the intensity of label per labelled cell. Intranuclear viral inclusion bodies were formed in as high as 90% of the hamster kidney cells infected with adeno-5, which replicates in hamster cells. The incorporated label was closely associated with the intranuclear viral inclusion bodies. No viral inclusion bodies were found in the hamster kidney cells infected with adeno-12, which has an incomplete growth cycle in hamster cells. *Hamster embryo cultures* contained only a small percentage of epithelial cells and it was only in these that the intranuclear viral inclusion bodies were found following infection with adeno-5. Fibroblastic cells, and therefore hamster embryo cultures, were much less susceptible to adenovirus-5 (and probably to adeno-12) than the predominantly epithelial hamster kidney cultures. The percentage of labelled cells following infection of hamster embryo cultures with either adeno-5 or adeno-12 increased only slightly as compared to the hamster kidney cultures.

That viral DNA is formed even in the

abortive infection of hamster cells with adeno-12 is suggested by a) decreased mitotic index but increased DNA synthesis induced by either adeno-5 or adeno-12, and b) association of the incorporated DNA-label with the viral intranuclear inclusions of hamster cells infected with adeno-5.

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Inhibtion of Endotoxin Fever by Reserpine.* (31242)

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The present studies demonstrate that treatment of rabbits with reserpine inhibits the febrile response to bacterial endotoxin injected 24 hours later, and explore the relationship of this inhibition to reserpine-induced depletion of brain stem 5-hydroxytryptamine (5HT) and norepinephrine (NE), both of which have been implicated in temperature control mechanisms(1,2).

Materials and methods. Adult male albino rabbits were trained to tolerate restraints and rectal temperature probes for 8-hour periods on at least 3 consecutive days. Temperatures were recorded every 10 minutes (Telethermometer, Yellow Springs Instrument Corp., Yellow Springs, Ohio) during the training period and for at least 3 hours before pyrogen challenge. Only animals achieving a stable baseline temperature were used. All experiments were performed in an inside, windowless room in which temperature varied little from 20°C. Temperature was charted on coordinate paper using scales of 4 cm per degree C on the ordinate and 3 cm per hour on the abscissa. The area between the baseline temperature and the fever curve over a 3-hour period following pyrogen challenge was determined by planimetric integration and expressed as the fever index (FI) in cm^2. An endotoxin of the Boivin type (Difco *E. coli* 0127 B:8) was obtained from commercial sources, suspended in pyrogen-free saline, and used in the indicated dosage. In general, fairly high dosage (25 μg) was employed to facilitate recognition of fever inhibition. Ani-

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