

Brief Definitive Reports

INDUCTION OF ARGINASE ACTIVITY WITH THE SHOPE PAPILLOMA VIRUS IN TISSUE CULTURE CELLS FROM AN ARGININEMIC PATIENT*

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Two cases of argininemia were described by Terheggen and associates (1). As the Shope rabbit papilloma virus induces arginase in rabbits and certain other animals including man (2), tissue culture cells from these individuals provide a model to find whether genetic information deficient in these patients could be replaced by information induced by and apparently coded for by a virus (3). The results of this study using cells from one of these individuals are described herein.

Materials and Methods

Skin fibroblasts derived by biopsy from one of the patients (A.W.) were cultured in vitro using standard tissue culture methods. Eagle's medium containing 15% fetal calf serum was used. Some flasks of the cells were inoculated with 0.5 mg of Shope virus that had been purified by rate banding in cesium chloride of a 1.29 density, washed by pelleting several times in physiological sodium chloride, and filtered using a 0.35 μ m Millipore filter (Millipore Corp., Bedford, Mass.). This virus preparation was found free of pleuropneumonia-like organisms and rabbit kidney vacuolating virus that are sometimes found contaminating Shope virus preparations (4); no cytopathic or other morphological effect was found in serial passage on rabbit kidney cells or upon normal or argininemic human cells. The amount of arginase activity in the medium or extracts of cells was determined by adding [14 C]arginine and isolating the radioactive ornithine product using standard amino acid analyzer techniques. Beckman 120C analyzers (Beckman Instruments, Inc., Fullerton, Calif.) were used. Measurement of [14 C]ornithine was made using a Packard Tri-Carb scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.).

Intact confluent monolayer cultures of A.W. fibroblasts in 75-cm² plastic flasks (Falcon Plastics, Division of BioQuest, Oxnard, Calif.), both normal and virus inoculated, were washed and replaced with 15 ml of Eagle's medium without serum. 10 μ Ci of [14 C]arginine (New England Nuclear, Boston, Mass.) was added to each flask. After 18 h incubation 2 ml of the cell-free medium was put on an amino acid analyzer column. The radioactive ornithine was collected and counted in the scintillation counter.

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RESULTS

Table I shows the relative counts derived from such cultures that were monolayers estimated microscopically to be of about equal size with and without added virus. It is evident that added virus increased the arginase activity as measured by the presence of [^{14}C]ornithine. When corrected for dilution, the number of counts is 70 times that indicated in the table where actual counts are shown.

Cell-Free Extracts.—Cell-free extracts of cultures that had been inoculated with virus or that were virus-free were compared. The cells of both sorts were washed three times with medium containing no calf serum and five times with physiological saline. They were extracted by freezing and thawing five times using a dry ice-alcohol bath. The cellular debris was centrifuged out and the supernate adjusted to 0.05 M manganese maleate at pH 7. This was incubated for 3 h to activate the arginase before diluting it with water to 0.001 M manganese. 0.5 ml of 0.05 M arginine at pH 9.5 and 10 μCi of [^{14}C]arginine were

TABLE I
Arginase Activity as Measured by the Radioactivity of [^{14}C]Ornithine Produced by A. W. Fibroblasts Incubated with [^{14}C]Arginine for 18 h in Culture after Inoculation of the Shope Virus

Sample no.	Counts per 10 min	
	Without virus	With virus
1	973	7,131
2	643	1,401

added to 10 ml of the diluted, activated extract that was then incubated for 60 min at 25°C. This is a standard method of testing for the presence of arginase activity (5). The cells extracted had been diluted 100 times at the time of test and the [^{14}C]ornithine 10 times in separation of ornithine collected from the columns. The cell extracts were tested with samples taken at zero time and 60 min, or at zero time, 30 and 60 min. The amount of activity is expressed per milligram of protein. The amount of protein was determined using the Lowry method (6). The arginase units were determined by the standard method of Greenberg (5) except that amounts of ornithine produced were measured rather than urea. An arginase unit is that amount that in 1 min at 25°C and pH 9.5 and with a substrate concentration of 0.285 M arginine will liberate 1 μmol urea or ornithine (5). In Table II the results of studies with the argininemic's cells, as well as a normal diploid human fibroblast cell line 290, originated by Dr. Ernest Chu of the Biology Division, Oak Ridge National Laboratory, are shown. In one of these tests of the argininemic's cells, trace amounts of arginase activity were found. In another test, however, none was detectable. This is in agreement with the previous studies of Terheggen and associates (7)

who found trace amounts of arginase activity in red blood cells of the patients with argininemia. The virus also increased arginase activity in the normal human cell line 290. The amount of arginase in crude extracts of liver is greatest as would be expected as the liver is the prime source of this enzyme. Upon testing it was found that the counts found in tests of the viral-free cells or at zero time in Table II are almost entirely due to [^{14}C]ornithine contamination of the [^{14}C]arginine used in the test system. This is corrected for in Table II.

It is recognized that the number of levels obtained was small. This is because of the extraordinarily slow growth of the cells that resulted in the small amount of protein extractable (Table II).

TABLE II
Specific Arginase Activity in Cell Extracts from A. W. Fibroblasts or Normal Human Diploid Fibroblasts (OR 290) Grown in the Presence or Absence of Shope Virus

Origin of cell extract	Protein	Time (min) of incubation			Arginase units per milligram of protein
		0*	30	60	
	<i>mg/cc</i>		<i>cpm</i>		<i>U/mg</i>
A.W. without virus	1.24	0		2,260‡	0.06
A.W. with virus	0.80	0		5,800	0.14
A.W. without virus	0.25	0	20	70	Trace
A.W. with virus	0.05	0	120	260	0.10
290 without virus	0.18	0		2,060	0.22
290 with virus	0.16	0		2,520	0.29
Hoise liver§					0.75

* Zero times counts corrected for [^{14}C]ornithine present in [^{14}C]arginine preparation. Counter accuracy within 3 cpm. Column reproducibility of ornithine isolation within 3% error.

‡ Counts per 10 min of [^{14}C]ornithine.

§ Greenberg, D. M. (5).

Fluorescent antibody studies were also made of these cells. Purified virus-induced arginase was derived from the papillomas of rabbits induced with the virus (8). This enzyme was then injected once a week intraperitoneally and subcutaneously into several rabbits for 3 wk, and then 1 wk later the rabbits were bled and their serum globulin was conjugated with the fluorescent dye (Sylvania Corp., Millburg, N. J.). Cells from A.W. with and without virus were grown out on cover slips, fixed in cold acetone, rinsed with physiological saline, dried and incubated in a closed, moist box for 1 h, and covered with a 1:10 dilution of the conjugated antiserum (9). One set of cover slips was incubated with the same serum but which had been absorbed with purified papilloma arginase. In Fig. 1 it is plain that there is much fluorescence of the cells derived from virus-infected cultures and little or none in the nonvirus-infected cells. This fluorescence was reduced in the virus-infected cells treated with the

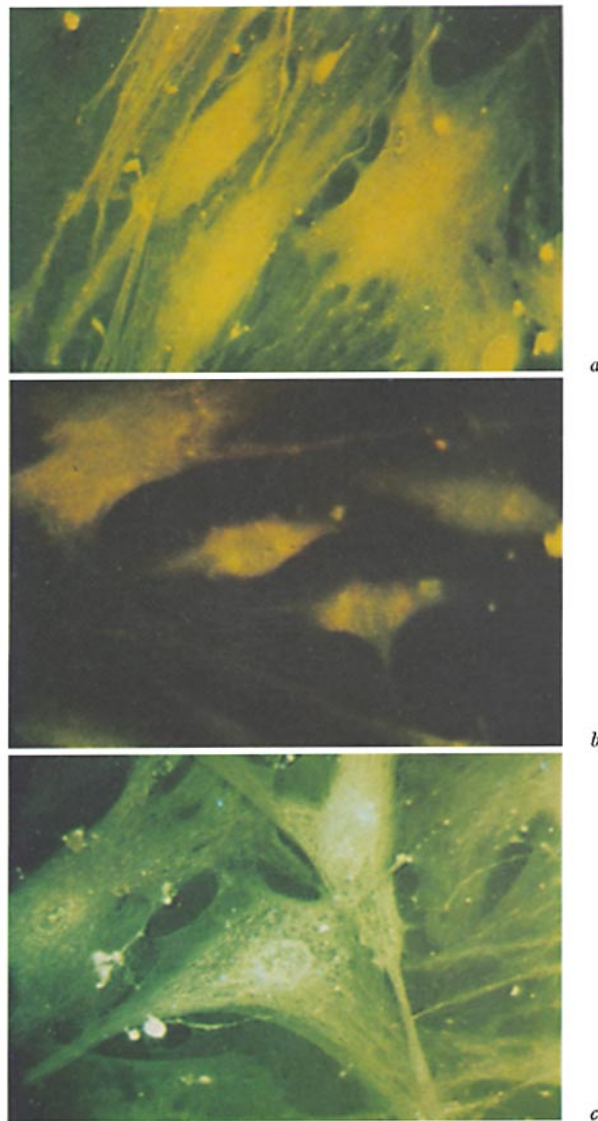


FIG. 1. (*a*) Shope virus-infected argininemic's cells exposed to specific antisera against Shope virus-induced arginase. (*b*) Shope virus-infected argininemic's cells exposed to same antiserum but which has been absorbed with purified arginase derived from Shope virus-induced papillomas. (*c*) Nonvirus-infected argininemic's cells exposed to antiserum against Shope virus-induced arginase. $\times 640$.

antiserum that had been absorbed against the virus-specific enzyme. As some fluorescence was seen in the nonvirus-infected cells, it is not possible to accurately quantitate the number of virus-infected cells. One's impression, however, is about half of the cells are virus infected. This rough proportion, taken

together with the results of Table II, suggest that were all the cells infected, the amount of arginase activity would approach that of the normal human fibroblasts. It is further noteworthy that the time since the inoculation of the cultures and the time of these activity tests and fluorescence studies is about 7 mo. The virus genome has not yet been lost during repeated subculturing of the cells.

DISCUSSION

These findings are of particular interest in relation to the studies previously reported of the serum levels of arginine in individuals who have worked in the laboratory with this virus. At least two people still had depressed blood arginine levels after an interval of about 20 yr from contact with the virus (2). The lack of any discernible cytopathic or other morphological effect on the cells in culture is also consistent with the lack of harmful effect upon people who acquired the virus through inadvertent laboratory infection. Infective virions have not been found in these cultures. This was most unlikely, however, as the cottontail rabbit from Kansas is the only known source of this virus.

It appears from these studies that the Shope virus should prove a useful adjunct to the therapy of the monogenic deficiency disease, argininemia.

SUMMARY

Inoculation of the Shope virus in tissue cultures of human fibroblasts from a patient with a deficiency of the enzyme arginase results in an induction of arginase activity, apparently virus coded.

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