PAPERS AND ORIGINALS

Human Polyomavirus Infection in Renal Allograft Recipients

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Summary

Cytological and virological studies on 74 patients with functioning renal allografts were undertaken to detect polyomavirus infection of the renal tract. Ten patients (13·5%) were excreting polyomavirus. Virus particles were seen in the electron microscope in urine samples from eight patients. B.K. polyomavirus was isolated from four patients. Infection with a different polyomavirus was probable in one patient. Virus isolation was most readily achieved when large numbers of intact virus particles were seen in the urine. Patients who were excreting large amounts of polyomavirus shed numerous inclusion-bearing cells which could be detected by cytology. A serological study showed that the prevalence of B.K. antibody was similar to that found in the general population and 38% of this series of transplant patients had evidence of active infection with B.K. virus.

Introduction

Viruses morphologically resembling members of the polyomavirus group have been recovered recently from man. The first of these, named B.K. virus, was isolated in tissue culture from the urine of a renal transplant patient (Gardner *et al.*, 1971). Three other viruses have been grown in tissue cultures inoculated with brain material from cases of progressive multifocal leucoencephalopathy, a rare neurological disease (Padgett *et al.*, 1971; Weiner *et al.*, 1972). One of these, the J.C. virus isolated by Padgett *et al.*, was shown by immunofluorescence to be unrelated to three members of the papovavirus group, polyoma, simian virus 40 ($S.V._{40}$), and human papillomavirus. The other two strains, however, isolated by Weiner *et al.*, were reported to be closely related if not identical with $S.V._{40}$, a virus found in kidney cell cultures of certain species of monkeys. B.K. virus was found to be unrelated to polyoma and human papillomavirus but had a minor antigenic relationship with $S.V._{40}$.

In the patient from whom B.K. virus was isolated numerous inclusion-bearing cells were noted in several of his urine samples and large numbers of polyomavirus particles were shown by electron microscopy. Serological studies suggested that the infection resulted from activation of latent virus during immunosuppressive therapy. As a result of these observations a cytological, serological, and virological study of patients with functioning renal allografts was made for further evidence of infection with human polyomaviruses, particularly B.K. virus. The results of these investigations are reported in this paper.

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Materials and Methods

STUDY GROUP

The study group consisted of 74 patients from St. Mary's Hospital, London, with functioning renal allografts; 71 patients were transplanted with cadaver kidneys and three were given kidneys from live related donors. Thirteen patients received two or more grafts and the mean survival time estimated from the date of the first transplant operation was 35·4 months. Altogether 45 patients were male and 29 female, and their ages ranged from 12 to 43 years (mean 27·3 years). All were receiving azathioprine 3 mg/kg body weight and prednisolone 15 mg daily. In addition 15 patients received cyclophosphamide 50 mg daily for varying periods during the course of this investigation, which continued for two years. Each patient was identified by a number at the time of transplantation. The same numbers have been used in previous publications (Pletka *et al.*, 1969; Hulme *et al.*, 1972).

CYTOLOGY AND ELECTRON MICROSCOPY

The techniques for the collection and preparation of urine samples for cytodiagnosis and for examination in the electron microscope were described in earlier communications (Gardner

et al., 1971; Coleman *et al.*, 1973). A slight modification of this procedure for electron microscopy was the occasional use of whole urine samples instead of clarified urine and 3% phosphotungstic acid (pH 6·0) instead of silicotungstic acid.

ISOLATION OF B.K. VIRUS

Urine samples were centrifuged at 1,000 r.p.m. for 10 minutes and 0·2 ml of the deposit and supernatant was inoculated separately into Vero and human embryo lung fibroblast (H.E.L.) cell cultures and incubated stationary at 37 °C.

The Vero cultures were maintained by weekly fluid changes and when possible were incubated for at least three months. At weekly intervals the monolayers were examined microscopically for the characteristic cytopathic changes produced by B.K. virus. These changes were described in a previous paper (Gardner *et al.*, 1971). At intervals during the period of incubation and before the cultures were discarded the culture fluids were examined for the presence of haemagglutinin using 0·5% human O cells.

The H.E.L. cell cultures were primarily used for the isolation of cytomegalovirus and were incubated for 28 days and maintained by twice-weekly fluid changes.

SEROLOGY

A total of 288 serum samples from 73 of the renal allograft recipients studied were available for testing for antibody to B.K. virus. The sera were obtained from the hepatitis serum bank of the Virus Reference Laboratory, Colindale, and from the tissue typing laboratory at St. Mary's Hospital. Several sera covering a long period were obtained from most patients.

The renal allograft recipients were placed in two groups. *Group* 1 consisted of 40 patients from whom the first serum was taken before the renal transplant operation. Further sera taken at least three months after operation were available from 36 patients. *Group* 2 contained 33 patients from whom the first serum was taken after the renal transplant operation. In some cases sera were collected only after an interval of several years. More than one serum was tested from each person in this group.

Initially the sera were examined for B.K. antibody using the techniques of complement fixation and haemagglutination inhibition. Though the results of preliminary studies showed a good correlation between the two methods used the complement fixation test was found to be less sensitive. The serological investigation was therefore continued using the haemagglutination inhibition technique only; details of the method used have been described in a previous paper (Gardner, 1973).

Results

CYTOLOGY AND ELECTRON MICROSCOPY

Millipore preparations of 255 urine samples from 74 patients were examined by light microscopy at low magnification (×100) for the presence of epithelial cells with large basophilic intranuclear inclusions. The inclusion-bearing cells were readily recognized at low magnifications by virtue of their large size and intense staining properties. The dense basophilic homogeneous inclusion body filled the nucleus, which was enlarged and occupied more than two-thirds of the cell. The nuclear membrane was clearly thickened by a heavy deposition of chromatin on the inner surface (fig. 1). The diameter of the inclusion-bearing cells ranged from 35 to 45 μm and they contrasted sharply with the normal transitional epithelial cells, which had an average diameter of 15 to 20 μm. The cytological pattern of infection with human polyomavirus is consistent with the histopathological appearance of the renal tract. This is characterized by pronounced proliferation of the transitional epithelium with large basophilic inclusions in the surface epithelial cells (Coleman *et al.*, 1973).

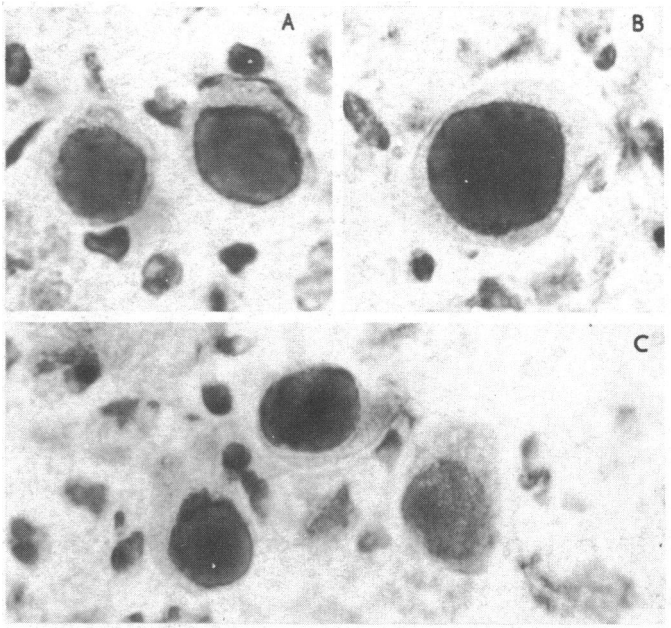

FIG. 1—Case 108. Three high-power fields of transitional epithelial cells in urine showing large intranuclear inclusions. (Haematoxylin and eosin. ×1,000.)

The large intranuclear inclusion-bearing cells seen in polyomavirus infection differed from the virus-infected cells described in cytomegalic inclusion disease in several respects. The nuclear/cytoplasmic ratio is unaltered in cytomegalovirus-infected cells and the cytoplasm is more abundant and may contain numerous small basophilic inclusions. The "bird's eye" appearance typical of nuclei infected with cytomegalovirus and due to a clear halo around the inclusion was not seen in the exfoliated polyomavirus-infected cells in our preparations.

The cytological results are shown in table I. Considerable variation in the number of cells with inclusions was found in different samples. Altogether 30% of the patients were excreting such cells.

TABLE I—*Incidence of Intranuclear Inclusion-bearing Cells in 255 Urine Samples from 74 Renal Allograft Recipients*

Cytological Findings	No. of Samples	No. of Patients
Numerous inclusions*	20	5 (7%)
Scanty inclusions†	29	17 (23%)
No inclusions	197	
Unsatisfactory sample	9	

* Five to 25 affected cells seen per low-power field.
† Only 2 or 3 affected cells per slide.

Altogether 112 urine samples from 50 patients were examined in the electron microscope, each grid being studied for at least 20 minutes. Samples from eight patients (16%) were found to contain polyomavirus particles on one or more occasions. No virus particles were seen in the remaining 97 samples from 42 patients.

The number of virus particles observed was usually small, with the notable exception of two urine samples from one patient (case 108) in which they were abundant. Virus particles were frequently coated with filamentous structures resembling antibody molecules (fig. 2). In four separate urine samples from one patient (case 124) the particles, while recognizably polyomavirus, were nearly all disrupted (fig. 3). The findings on the eight positive individuals are detailed in table II.

Fourteen urine samples which contained numerous inclusions and came from five patients (table I) were examined in the

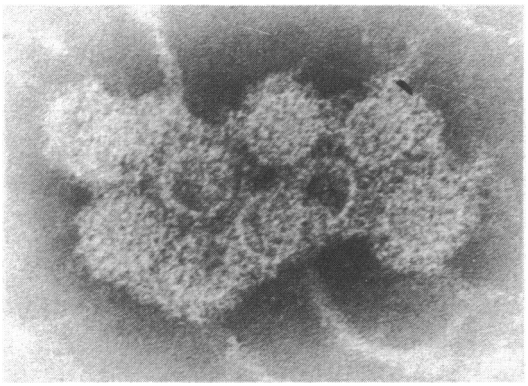

FIG. 2—Case 123. Polyomavirus particles in urine. Particles are coated with filamentous structures resembling antibody molecules. (×240,000.)

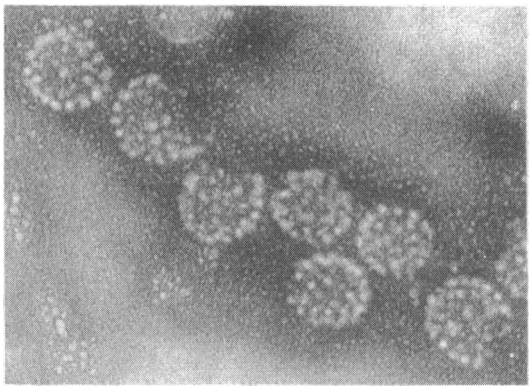

FIG. 3—Case 124. Intact and disrupted polyomavirus particles in urine. (×240,000.)

TABLE II—*Findings by Electron Microscopy on Urine Samples from Patients excreting Polyomavirus*

Case No.	No. of Urine Samples Examined	No. of Urine Samples with Polyomavirus	No. and Appearance of Polyomavirus Particles
140	2	1	Few particles
MLD 8	5	1	One particle, heavily coated*
123	5	3	Moderate number of particles, heavily coated
60	1	1	Two particles, heavily coated
160	5	2	Few particles, some heavily coated
108	2	2	Many particles. In second sample a few were lightly coated. In both samples many particles were closely associated with membranes among cell debris
124	4	4	Moderate number of particles, many disrupted
127	2	1	Few particles, lightly coated

* Coated = antibody-like structures attached to virus particles.

electron microscope. Polyomavirus particles were seen in 11 samples from all five persons. Electron microscopical studies were also carried out on 14 urine samples with scanty inclusions from 13 patients. No polyomavirus particles were seen.

No herpesvirus (cytomegalovirus) particles were seen in any urine sample by electron microscopy.

VIRUS ISOLATION

A total of 118 urine samples from 57 patients were examined in Vero cell cultures. Cultures inoculated with 92 of the samples (78%) were incubated for a period of three to eight months. The remaining 26 were incubated for two months only. A virus antigenically identical with B.K. virus and having similar biological properties was isolated from five urine samples from four patients (7%).

The correlation between electron microscopy and cytology results on these five samples and the period of incubation necessary in Vero cells before cytopathic changes and haemagglutinin production occurred is shown in table III. Large numbers of polyoma-like virus particles were seen by electron microscopy in two urine samples from case 108. B.K. virus was isolated from both samples after an incubation period of two and three months respectively. The second urine sample was collected 11 days after the first. The first sample from this patient was also inoculated into a different line of Vero cells, which were later shown to be less sensitive, since B.K. virus was isolated in them only after an incubation period of four months.

TABLE III—*Correlation of Period required for Virus Growth with Electron Microscopy (E.M.) and Cytology of five Urine Samples*

Case No.	Inclusions Present	Polyomavirus Particles Present by E.M.	Incubation Period in Vero Cells required for Growth of Virus
MLD 8	None	Single particle	4 Months
148	None	None	5 Months
108 (1st sample)	Numerous	Large numbers	2 Months
108 (2nd sample)	Numerous	Large numbers	3 Months
134	None	None	5 Months

B.K. virus was also isolated from case 108 by direct inoculation of the second urine in H.E.L. cell cultures. After an incubation period of about three months a granular degeneration of the fibroblast cells was seen, which on passage was quite distinctive.

Cytomegalovirus was isolated from 35 (61%) of the 57 patients investigated.

INCIDENCE OF ANTIBODY TO B.K. VIRUS

The number of patients with B.K. antibody in the first serum is shown in table IV. The incidence was similar in the two groups but the proportion with high titres was much greater in group 2.

TABLE IV—*B.K. Antibody in First Serum from 73 Renal Transplant Patients detected by Haemagglutination Inhibition (H.I.)*

Group	No. of Patients	No. with Antibody	No. without Antibody	H.I. Titre 2,560 or Greater
(1) First serum taken before transplantation	40	28 (70%)	12 (30%)	2 (5%)
(2) First serum taken after transplantation	33	25 (76%)	8 (24%)	16 (48%)
Total	73	53 (73%)	20 (27%)	18 (25%)

B.K. ANTIBODY RESPONSE TO IMMUNOSUPPRESSIVE THERAPY

B.K. antibody response was studied in 69 patients while they were receiving immunosuppressive drugs. The results are shown in table V. Active infection with B.K. virus as judged by changes in antibody titres was clearly evident in 20 of the 36 patients (55·5%) in group 1. Four of these 20 patients had no detectable antibody in early sera but became positive after transplantation. Of 16 of the 20 patients who had antibody at the time of transplantation a significant rise was shown in 13. In most cases the rise was substantial and permanent. In three patients a significant fall in titre was shown.

TABLE V—*B.K. Antibody Response during Immunosuppressive Therapy*

Group	No. of Patients	Significant Change in Antibody Titre	No Change in Antibody Titre
(1) First serum taken before transplantation	36	20 (55·5%)	16 (44·4%)
(2) First serum taken after transplantation	33	6 (18%)	27 (82%)

In contrast only 6 (18%) of the 33 patients in group 2 had significant changes in antibody titres. Four of the six patients had negative to positive seroconversions and the other two developed significant increases in titre. Many of the patients in group 2 had high haemagglutination inhibition antibody titres in the first sera (table IV). In most cases these serum samples were collected late after the transplant operation, by which time the antibody levels were probably permanently high. This may be the reason why relatively few significant changes in antibody titres were found in group 2 compared with group 1 (table V).

Eight patients with no detectable B.K. antibody in the first serum developed haemagglutination inhibition antibody while receiving immunosuppressive drugs (table VI). Two of these patients (cases 106 and 123) produced only a minimal antibody response, which was not maintained. In both cases the antibody titres later fell to 1 in 20. This is the type of antibody response which might be expected when a primary infection occurs in an immunosuppressed patient, but it is also possible that this change in titre was the result of an anamnestic response in a patient infected with an antigenically related polyomavirus. Case 123 was shown by electron microscopy to have polyomavirus particles in his urine but B.K. virus was not isolated. Five of the remaining six patients showed a substantial B.K. antibody response. It may be in these patients that this was the result not of a primary infection but of reactivation. Low levels of antibody not detected by the haemagglutination inhibition test used may have been present in the first serum.

TABLE VI—*Development of Haemagglutination Inhibition (H.I.) Antibody to B.K. Virus during Immunosuppressive Therapy*

Case No.	Age (Years)	Time relative to Transplantation (Months)	Serum H.I. Antibody Titre
167	35	−3	<20
		+9	640
147	34	−2	<20
		+21	1,280
106	16	−3	<20
		+1	320
		+15	20
		+4	<20
		+10	<20
		+12	20
123	30	+13	160
		+15	80
		+16	80
		+33	20
173	31	−1	<20
		+6	320
161	29	+5	<20
		+10	2,560
154	32	+1	20
		+18	20,480
		+1	<20
128	43	+2	2,560
		+12	2,560
		+29	2,560

POLYOMAVIRUS EXCRETION IN 10 PATIENTS

Of the 74 renal transplant patients studied 10 (13·5%) were found to be excreting polyomavirus by two or more methods of investigation. One patient (case 108) was positive by all methods. The results of the investigations are related in table VII. It was not possible to correlate excretion of the virus with the clinical condition of the patient. Polyomavirus was excreted over a period of five months in case 123 and for 13 months in case 124.

Three of the patients who were excreting polyomavirus died during the period of study. Many inclusion-bearing cells were seen in histological sections of postmortem material in one patient only (case 108) who died from septicaemia and from whom B.K. virus was isolated on two occasions during life. Intranuclear inclusions were noted in lung, liver, spleen, and bone marrow and were scattered throughout the renal tract. Inclusions were particularly numerous in the wall of a subphrenic abscess and in adjacent bowel. Cytomegalovirus but not B.K. virus was isolated from many organs. Cytomegalovirus was also isolated from postmortem material from the other two patients (cases 60 and 160).

TABLE VII—*Correlation of Observations on Human Polyomavirus Infection in 10 Renal Transplant Patients*

Case No.	Numerous Inclusions by Light Microscopy	Polyomavirus Particles by Electron Microscopy	B.K. Virus Isolation	Significant Rise in B.K. Antibody
140	+	+	Not completed	+
MLD 8	−	+	+	+
148	−	−	+	+
123	+	+	−	+
60*	+	+	−	−
160*	−	+	−	+
108*	+	+	+	+
124	+	+	+	+
134	−	−	+	+
127	−	+	−	+

*Patient died.

Discussion

In this study the methods of exfoliative cytology, electron microscopy, and virus isolation were applied to the detection of a new virus infection. Of the 74 renal allograft recipients investigated over a period of two years 10 (13·5%) were found to be excreting polyomavirus from the renal tract. In four of these patients virus was isolated and identified as B.K. virus. In seven out of eight virus excretors identified by electron microscopy a significant antibody rise to B.K. virus was shown. In the eighth patient (case 60), however, no B.K. antibody was detected. Inclusion-bearing cells were noted in the urine but no virus was isolated. These findings suggested that infection of the renal tract with more than one kind of polyomavirus could occur and the virus seen in this patient may have been one of the other members of the group.

Though only 13·5% of the transplant patients were identified as excretors serological studies showed that infection of renal transplant patients with B.K. virus was probably more frequent. Twenty-six out of 69 patients (38%) had a significant change in B.K. antibody titre, which suggested an active infection with this virus. An antibody survey of the general population (Gardner, 1973) has shown that primary infection with B.K. virus occurs mainly in childhood and antibody is common in the adult population. The transplant patients did not differ from the normal population in the incidence of antibody. Since more than 70% of the patients studied had B.K. antibody before receiving a kidney it seems likely that reactivation of latent virus during immunosuppressive therapy was responsible for the change in antibody titres. Nevertheless, reinfection with B.K. or another antigenically related human polyomavirus cannot be excluded.

A recent report from South Africa (Lecatsas *et al.*, 1973) described polyomavirus particles in the urine of 8 (44%) out of 18 renal allograft recipients. This incidence is higher than in our series, where using smaller volumes of urine and fewer specimens per patient polyomavirus was seen in only 8 (16%) out of 50 patients.

The situation in which polyomavirus was seen in the urine yet not isolated in tissue culture may be explained in several ways. Sometimes the urine was not cultured for the length of time necessary to grow B.K. virus (cases 123 and 160). The line of Vero cells occasionally used was subsequently found to be comparatively insensitive for B.K. virus isolation. Often the virus particles in the urine were coated with an antibody-like substance which may have neutralized the infectivity of the virus. It is also possible that the particles seen were a different polyomavirus which could not grow under the culture conditions used. A further reason why visible virus might not grow in cell culture is exemplified by case 124. The particles seen in the urine samples were mostly broken open and appeared incomplete. A significant rise in B.K. antibody by complement fixation and haemagglutination inhibition was shown in this patient but despite several attempts to isolate B.K. virus from these urine samples no virus was grown even after a seven-month incubation period, probably because of the defective nature of the particles.

From a practical point of view cytology is a convenient screening method for detecting possible polyomavirus excretors. Electron microscopy is more sensitive but less practical for large surveys. Virus isolation presents the difficulty of maintaining large numbers of cultures for long periods of incubation. Cytology can detect strongly positive excretors such as case 108, and it is from such patients that virus is most readily isolated. In large surveys it may be appropriate to use cytology as a preliminary screening procedure with electron microscopy and virus isolation for confirmation of virus excretion.

The only human disease with which polyomaviruses have so far been associated is the rare neurological disorder progressive multifocal leucoencephalopathy (P.M.L.). Patients with this disease are often found to have immunological defects, and a single occurrence of P.M.L. has been described in a patient 17 months after renal transplantation (Manz *et al.*, 1971). No case of P.M.L. occurred in our series of patients. They will continue under observation because of the possibility of P.M.L. developing and because of the oncogenic potential of this group of viruses.

We are grateful to Professor W. S. Peart and Dr. B. Hulme for permission to investigate this group of patients, and to Mr. A. A. Porter for help with the electron microscopy.

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Vasopressin Analogue DDAVP in Diabetes Insipidus: Clinical and Laboratory Studies

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Summary

In seven patients with cranial diabetes insipidus an analogue of vasopressin, DDAVP, produced an antidiuresis lasting up to 20 hours after a single intranasal dose. Lysine vasopressin (LVP) in the same dose produced a less potent antidiuresis which lasted for only three to four hours. The plasma half life of DDAVP was 7·8 and 75·5 min for the fast and slow phases, compared with 2·5 and 14·5 min for LVP. Radioiodine-labelled DDAVP was not destroyed by incubation with late pregnancy plasma, which contains an enzyme that inactivates vasopressin. The slow metabolic clearance of DDAVP, its absorption through the nasal mucosa, and its lack of side effects make this the ideal drug for the treatment of vasopressin-sensitive diabetes insipidus. Patients usually require 10 to 20 μg DDAVP given intranasally twice daily for good clinical control of their diabetes insipidus.

Introduction

Though many different forms of therapy have been used in the treatment of diabetes insipidus (D.I.), until recently injections of Pitressin Tannate (vasopressin tannate) in oil have been the only satisfactory method for the treatment of severe cases. Pitressin, however, is an impure preparation which includes several peptides other than vasopressin, including oxytocin, neurophysin (Martin, 1971), prolactin, and a corticotrophin-like peptide (Scott *et al.*, 1972). Martin (1971) showed that patients treated with Pitressin may produce high titres of antibodies against neurophysin. Furthermore, patients often find the injections difficult to administer and unpleasant to receive.

A number of drugs unrelated to vasopressin have been found to be useful in the management of diabetes insipidus, including diuretics (Crawford and Kennedy, 1959), chlorpropamide (Arduino *et al.*, 1966), carbamazepine (Braunhofer and Zicha, 1966), and clofibrate (De Gennes *et al.*, 1970). The mechanism of their action is not well understood, but chlorpropamide appears to act by increasing renal responsiveness to endogenous vasopressin (Berndt *et al.*, 1970; Miller and Moses, 1970). Side effects such as hypokalaemia with diuretics and hypoglycaemia with chlorpropamide have limited their use, especially in children. Furthermore, for effective chlorpropamide therapy some functioning posterior pituitary must remain, and in many patients the "Antabuse"-like actions of chlorpropamide are unacceptable.

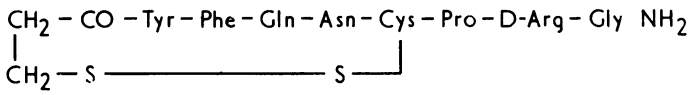

FIG. 1—Amino-acid sequence of (1-desamino-8-D-arginine) vasopressin, DDAVP. Also known as (1-beta-mercaptoproprionic acid -8-D-arginine) vasopressin.

Synthetic lysine vasopressin (LVP) given as a nasal spray has advantages over pituitary snuff in that it does not result in local hypersensitivity phenomena (Pepys *et al.*, 1965) or pulmonary complications (Mahon *et al.*, 1967). Its effects, however, are too short-lasting to be useful in patients with severe diabetes insipidus. An analogue of vasopressin, 1-desamino-8-D-arginine vasopressin (DDAVP) (fig. 1), has greater antidiuretic potency, longer duration of action, and much less pressor effect when compared with other commercially available preparations of vasopressin. Its synthesis and chemical properties were described by Zaoral *et al.* (1967) and a study of its pharmacological properties with a preliminary report of its clinical application by Vavra *et al.* (1968). It has been suggested that the prolonged

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