any such particles, and neither did the supernatant of the high-speed centrifugate. Thus the active principle of vibriocin cannot be related to the small particles discussed by Bradley[5]. It seemed reasonable to conclude, however, that the particles found in the biologically active fractions (partially purified material) and the pellet (purified material) were the inhibitory particles of vibriocin.

In Fig. 2*B* the fine structure of vibriocin particles can be seen. They seem to consist of double hollow cylinders 1100 Å long with an outer sheath and an inner core. The sheath is approximately 240 Å wide and the diameter of the core is 90–100 Å. The width of the inner space of the core measures 45–50 Å. Most of the particles consist of contractile rod-shaped forms; however, extended sheaths measuring only 200 Å wide are also seen (Fig. 2*C*). For comparison, vibrio phage of group IV propagated on the vibriocinogenic strain, is shown in Fig. 2*D*.

Thus, vibriocin has a contractile sheath and an inner core which seems to be empty in some particles and full in others (Fig. 2*A*). Bradley and Dewar also saw solid or empty cores for pyocin[5]. The inner space of the core may contain some material, possibly nucleic acid. Vibriocin[6] and other bacteriocins[5,7] are sensitive to proteolytic enzymes, but this may not exclude a possible biological function for the nucleic acid in these particles.

Vibriocin has been found to require an active oxidative phosphorylating and protein synthesizing cell in order to exert its activity. The relationship of vibriocin to virulence now being investigated has been found to be distinct from the ion-translocase inhibitor described by Richardson and Evans[8].

We thank Professor Frances Doane and Miss Nan Anderson for carrying out the electron microscopy. One of us (A. J.) thanks the Medical Research Council for a fellowship.

ASOKA JAYAWARDENE
HANNAH FARKAS-HIMSLEY
Department of Microbiology,
School of Hygiene,
University of Toronto,
Toronto, Canada.

Received November 28, 1967; revised April 17, 1968.

[1] Farkas-Himsley, H., and Seyfried, P. L., *Nature*, **193**, 1193 (1962).
[2] Nicolle, P., *Rev. Hyg. Med. Soc.*, **10**, 91 (1962).
[3] Barua, D., *Nature*, **200**, 710 (1963).
[4] Wahba, A. H., *Bull WHO*, **33**, 661 (1965).
[5] Bradley, D. E., and Dewar, C. A., *J. Gen. Microbiol.*, **45**, 399 (1966).
[6] Farkas-Himsley, H., and Seyfried, P. L., *Abl. Bakt. I. Abt. Orig.*, **196**, 298 (1965).
[7] Ishi, S., *J. Mol. Biol.*, **13**, 428 (1965).
[8] Richardson, S. H., and Evans, D. J., *Bact. Proc. Amer. Soc. Microbiol.*, M 17, 41 (1966).

Oncogenicity of Mixtures of Adeno-associated Virus and Adenovirus Type 12

THE presence of adeno-associated virus (AAV) in certain stocks of various adenovirus types is well documented[1–4]. These viruses (four known serotypes) depend on adenovirus for replication, but at the same time depress the production of infectious adenovirus and virion antigens[2].

At present we are engaged in oncogenicity screening of all of the thirty-one types of human adenovirus in collaboration with Dr Maurice Green, St Louis University Institute of Molecular Virology. Recent tests have indicated that a number of the virus stocks, from which inocula were prepared, contained substantial quantities of either AAV type 1 or type 2. Although the preparations used for animal inoculation were purified by density gradient centrifugation, this procedure does not completely eliminate AAV from adenovirus bands. The experiments reported here were designed to establish whether AAV in substantial excess of that expected in purified adenovirus preparations affects the oncogenicity of a potent oncogenic adenovirus—type 12. Clearly, any depression in activity of a known oncogenic virus would seriously affect the interpretation of results obtained with weak or "non-oncogenic" viruses.

AAV types 1 and 2 (average density 1·40 g/ml.) were purified and separated from adenoviruses (average density 1·34 g/ml.) by banding three times in CsCl gradients. The final preparations, after removal of CsCl by dialysis, gave specific complement fixation (titres of 1 : 128–1 : 256 against AAV antibody and did not react with adenovirus antibody. Such preparations inoculated with Freund's adjuvant into guinea-pigs induced AAV antibody (titres of 1 : 1280) and no adenovirus antibody ($< 1 : 10$). According to Smith *et al.*[4], these AAV preparations should contain approximately 10^{11} AAV particles/ml. This is based on a particle : complement fixation ratio of approximately 10^9. (Biological activity of the AAV preparations, as determined by induction of complement fixation antigen in human embryonic kidney cells (HEK) using "clean" adenovirus type 7 as helper, was verified in separate experiments.) The adenovirus 12 pool (Huie strain) used in these experiments did not contain AAV, at least in amounts detectable by complement fixation tests with cell packs of infected HEK or KB cells. Adenovirus 12, $10^{7\cdot2}$ $TCID_{50}$/ml., was mixed with an equal volume of varying dilutions of either of the two AAV serotypes containing 10^8–10^{10} (estimated from complement fixation titres) particles/ml. Earle's balanced salt solution was used as diluent for the AAV and also as the control diluent. Newborn hamsters were inoculated subcutaneously with 0·1 ml. of the mixtures. Beginning 28 days after inoculation, the animals were palpated daily. The day of tumour incidence was based on the palpation of masses approximately 5 mm in diameter.

In previous experiments with this particular adenovirus 12 pool, the tumour first appeared between the thirty-second and thirty-eighth days after inoculation. Induction of tumours was found to be dependent on dose over at least a ten-fold concentration range so that a 10^{-1} dilution gave only 5–10 per cent tumours within an interval of 60 days. Even a 1 : 2 dilution of this pool gave a significant reduction in the incidence of tumours and an increased latent period, the earliest tumours being recorded 40–45 days after inoculation.

Table 1. ONCOGENICITY OF ADENOVIRUS 12—AAV MIXTURES

AAV concentration*	Tumours/survivors 55 days after inoculation	
Type 1 10^{10}	3/7	
10^{9}	2/2	9/14
10^{8}	4/5	
Type 2 10^{10}	2/3	
10^{8}	2/5	6/11
10^{6}	2/3	
Control—No AAV		7/12

* Based on an assumed particle : complement fixation ratio of 10^9—adenovirus constant at $10^{7\cdot2}$ $TCID_{50}$/ml.

The results of the mixing experiments (Fig. 1, Table 1) show that neither AAV serotype had any effect on the oncogenicity of adenovirus 12. There were no detectable differences in either time of onset or tumour incidence. In other experiments, dilutions of adenovirus 12 were mixed with the highest concentration of AAV (10^{10} particles/ml.). Only small numbers of animals have been tested; but again, no differences in the time of onset or incidence have been noted. The nature of the bioassay does not permit the discrimination of small effects; for example, 20 per cent in the oncogenicity of a given virus. Such effects, however, would not affect our main conclusion, namely, that inclusion of AAV in adenovirus inocula (within the concentration range reported here) would not be expected to influence oncogenicity. It should be understood that this conclusion

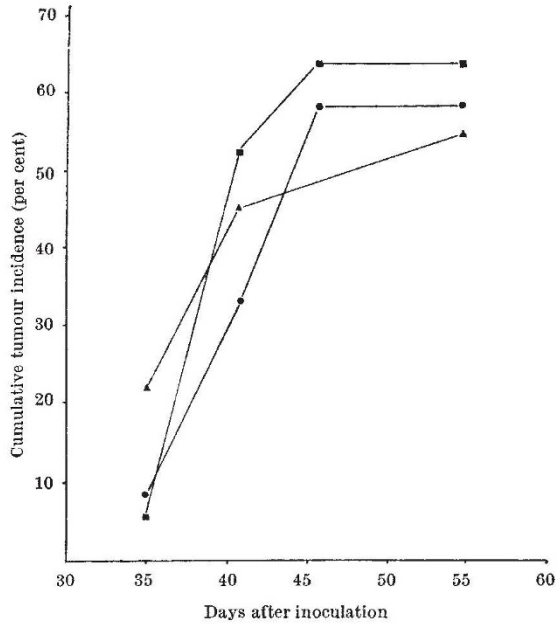

Fig. 1. Time course of tumour development for all animals inoculated with: (*a*) Ad 12 ●—●; (*b*) Ad 12+AAV−1 ■—■; (*c*) Ad 12+AAV−2 ▲—▲. Pooled data from Table 1.

is restricted to *in vivo* conditions and may not apply to *in vitro* transformation studies.

The results of extended screening of all the human adenovirus types (unpublished data) have so far shown that eight of the thirty-one types are oncogenic[5]. Certain of the types comprising the B group of adenoviruses (3, 7, 14, 16, 21) induce tumours with low frequency, 0·5–10 per cent and after long latent periods, up to 442 days. Such tumours can be designated as induced by virus, based on the presence of specific T antigens[5]. So far, the failure of the remaining twenty-three types to induce tumours in hamsters can be attributed to the intrinsic properties of the interaction of the virus-assay system and, from our results, presumably not to the chance inclusion of AAV in the purified virus preparations. Studies with mixtures of AAV and weakly oncogenic adenoviruses will be necessary in order to test this general conclusion.

We thank Mrs Edda Twiddy for technical assistance. This work was supported by the Vaccine Development Branch, National Institute of Allergy and Infectious Diseases, and by the Viral Carcinogenesis Branch, National Cancer Institute, US National Institutes of Health.

Raymond V. Gilden
Jerome Kern
Theodore G. Beddow

Flow Laboratories Incorporated,
Rockville, Maryland.

Robert J. Huebner

Laboratory of Viral Diseases,
National Institute of Allergy and Infectious Diseases,
National Institutes of Health,
Bethesda, Maryland.

Received March 11, 1968.

[1] Atchison, R. W., Castro, B. C., and Hammon, W. McD., *Science*, **149**, 754 (1965).
[2] Hoggan, M. D., Blacklow, N. R., and Rowe, W. P., *Proc. US Nat. Acad. Sci.*, **55**, 1467 (1966).
[3] Melnick, J. L., Mayor, H. D., Smith, K. O., and Rapp, F., *J. Bacteriol.*, **90**, 271 (1965).
[4] Smith, K. O., Gehle, W. D., and Thiel, J. F., *J. Immunol.*, **97**, 754 (1966).
[5] Huebner, R. J., *Persp. in Virol.*, **4**, 142 (1965).

Measurements of Very Small Osmotic Pressures of the Haemocyanin of *Pila leopoldvillensis*

Osmotic pressure measurements of solutions of *Pila* haemocyanin have been made with a toluene osmometer provided with a siphon tube[1]. With this apparatus, after osmotic equilibrium has been attained, the manometer can be connected with the dialysate by opening a screw-clamp, to give a reading of zero pressure. After reclosing the clamp and allowing osmotic equilibrium to be established again, a second reading of the osmotic pressure can be taken, and these procedures can be repeated as often as is required. If the molecular weight of the protein is to be calculated from osmotic pressure determinations, it is desirable to include measurements made on dilute protein solutions. A reading microscope has been used for measurements from 0·13 to 3 mm of toluene. Maximum and standard errors of readings are recorded in Table 1, together with osmotic pressures observed for solutions of haemocyanin equilibrated at 1° C with acetate buffers of ionic strength 0·15 and *p*H 5·2 and 5·94 and of ionic strength 0·02 and *p*H 5·90.

Table 1. OSMOTIC PRESSURES OF HAEMOCYANIN OF *Pila leopoldvillensis* IN SODIUM ACETATE BUFFERS

C g of protein in 100 ml. of solution	π osmotic pressure mm toluene at 1° C	Standard error	Maximum error	No. of measurements of π
*p*H 5·20		Ionic strength of buffer 0·15		
0·659	0·207	0·010	0·022	13
1·130	0·416	0·010	0·019	5
1·590	0·590	0·005	0·007	4
1·750	0·749	0·022	0·043	8
2·398	0·948	0·027	0·041	4
*p*H 5·94		Ionic strength of buffer 0·15		
0·415	0·132	0·009	0·018	8
0·428	0·149	0·007	0·017	13
0·616	0·205	0·009	0·016	5
1·139	0·378	0·011	0·018	5
1·639	0·637	0·010	0·026	59
2·387	1·004	0·007	0·011	4
*p*H 5·90		Ionic strength of buffer 0·02		
0·509	0·287	0·020	0·047	10
1·019	0·617	0·004	0·014	4
2·008	1·458	0·009	0·015	5

Methods of calculation of molecular weights of proteins from osmotic pressure measurements have already been described[1], and it has been emphasized that it is desirable to compare the results of alternative methods[2].

The mean value of the molecular weights calculated from measurements given in Table 1 is 8,600,000 for the solutions of ionic strength 0·15. The largest deviation from the mean value was 7 per cent and the mean deviation was about 3 per cent. This value agrees, within the limits of error, with the value of 8,756,000 obtained by the method of light scattering[3]. This agreement provides evidence for the homogeneity of the haemocyanin preparations. If 1 per cent of a substance of particle size 80,000 had been present, the osmotic pressures would have been twice as great as the values obtained.

Calculations of molecular weight from measurements made on solutions of ionic strength 0·02 (Table 1) confirm the results, obtained by zone electrophoresis[4], which have shown that at *p*H 5·90 and ionic strength 0·02, there has been considerable dissociation of the haemocyanin molecule.

Despite the very small osmotic pressures which require the use of a reading microscope and precautions to avoid temperature fluctuations at times when readings are made, *Pila* haemocyanin has advantages for osmotic pressure studies. It is very stable at 1° C; in one experiment fifty-nine measurements of pressure were made during 4 months. The largest deviation from the mean pressure observed was 0·026 mm of toluene. Collodion membranes of high permeability have been used, enabling osmotic equilibrium to be achieved rapidly, but the constancy of