A Comparison of Adenovirus 12 Induced T and Tumour Antigens by Rate-zonal Centrifugation

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SUMMARY

The sedimentation properties of adenovirus 12 induced antigens were studied by rate-zonal centrifugation in linear sucrose gradients. Adenovirus 12 hexon and fibre antigens were estimated to have molecular weights of 22–25 x 10^4 and 6–7 x 10^4 daltons, respectively. In addition, two species of T antigen were separated by rate-zonal centrifugation. The two T antigen species had molecular weights of 8–9 x 10^4 and 4–5 x 10^4 daltons and were distinct from adenovirus 12 hexon and fibre antigens. Results were identical whether the adenovirus 12 antigens were extracted from infected H.Ep-2 or HEK cells. Similar studies of tumour antigen from transplanted adenovirus 12 induced tumours in hamsters and CBA mice, and two hamster cell lines grown in vitro, revealed the presence of two species of tumour antigen. Molecular weight estimates for the two species of tumour antigen indicated that these antigens were similar in molecular weight to the T antigens. Differences in the relative proportions of the two species of antigen in tumour extracts and in T antigen extracts were found. Treatment of tumour extracts with sodium deoxycholate or ribonuclease did not alter the sedimentation properties of the tumour antigens.

INTRODUCTION

The cells of tumours induced in laboratory animals by the inoculation of adenovirus 12 contain a virus-specific tumour antigen (Huebner et al. 1963). A serologically identical antigen, termed T antigen (Huebner, 1966), has been identified in adenovirus 12 infected tissue cultures (Hoggan et al. 1965). Although little is known of the nature and function of T and tumour antigens, immunofluorescence studies have shown that they are predominantly intranuclear proteins produced earlier in infected cells than virus capsid antigens. In addition, the production of T antigen is not inhibited by the presence of some substances which prevent virus replication, such as 5-fluoro-2-deoxyuridine (Huebner, 1967). Comparable tumour and T antigens have been described for most of the DNA viruses which cause tumours (Sabin & Koch, 1964; Huebner, 1967; Gilden et al. 1968; Potter & Oxford, 1969).

Studies of adenovirus 12 induced antigens by rate-zonal centrifugation in linear sucrose gradients have indicated the presence of up to four species or molecular forms of T antigen. Tavitian et al. (1967), Gilden (1968) and Gilead & Ginsberg (1968) each identified only one molecular form and estimated the molecular weight to be 15, 4 to 5 and 2·0 to 2·5 x 10^4 daltons, respectively. However, Hollingshead et al. (1967),
identified two molecular species of antigen while Tockstein et al. (1968) reported the presence of four molecular forms of T antigen. Although the differences in the published estimates of the molecular weights of adenovirus T and tumour antigens may be due to technical variations, it is not clear why the number of molecular forms should vary. Some of the above results have been obtained for adenovirus 12 tumour antigen and some for T antigen, and it is not known if the molecular form of the antigen is the same in both cases. In addition, studies of adenovirus 12 T and tumour antigen have been carried out using extracts from cells of different species. A further study of these antigens by rate-zonal centrifugation is described using T antigen produced in two human cell lines infected with adenovirus 12 and tumour antigens obtained from four sources.

**Materials and Methods**

*Tissue cultures.* H.Ep–2 and human embryo kidney (HEK) cells were cultured in Eagle's minimal essential medium (MEM) containing 10% inactivated calf serum and antibiotics (100 units/ml. of penicillin and 100 μg./ml. of streptomycin), and maintained in MEM containing 2% calf serum and antibiotics. HEK cells were used as primary or secondary cultures. Two lines of adenovirus 12 induced hamster cells, the H212 and T.AdIII cell lines, both established from tumours induced in vivo and passaged over 150 times in the laboratory, were used. The H212 cell line (Schild, Oxford & Potter, 1968) had lost the property of transplantability to weanling and adult hamsters but produced tumours when inoculated into newborn hamsters. The second line, the T.AdIII cell line (Lorans, 1966), was obtained from Dr L. Berman, Inst. de Recherches Scientifiques sur le Cancer, Villejuif, France. This cell line was initiated in 1963 and has retained the property of transplantability in adult hamsters. The tumour cell lines were cultured in MEM containing 10% calf serum and antibiotics, and subcultured at 4 to 6 day intervals.

*Viruses.* A strain of adenovirus 12, isolated and serially propagated in HEK cells, was used to infect monolayer cultures of H.Ep–2 cells at a multiplicity of 1 to 5 TCD₅₀/cell. When full cytopathic effect was observed, commonly after 3 to 4 days, the cultures were frozen and thawed twice, the fluids pooled and centrifuged at 800 g for 20 min. The supernatant fluids were concentrated tenfold using a Diaflo ultrafiltration apparatus with a UM-10 membrane (Amicon Corp.,Lexton, Mass.) and a positive pressure of 24 psi (Blatt et al. 1967). The virus pool contained 10⁸–¹⁰ TCD₅₀/ml when titrated in HEK cultures (21-day end-point), and was free from complement-fixing antigens of adeno-associated virus types 1 to 4.

*Antisera.* Specific rabbit antisera to adenovirus 12 hexon and fibre antigens (Ginsberg et al. 1966) were kindly supplied by Dr. H. G. Pereira, National Institute for Medical Research, Mill Hill, London, N.W.7. A pool of serum from hamsters bearing large (> 50 mm. diameter), transplanted adenovirus 12 tumours (tumour hamster serum) was used to detect adenovirus 12 induced T and tumour antigens. This serum was 'narrow-reacting' (Huebner, 1966), and contained complement fixing (CF) antibody at a titre of 1:128 when tested against four units of adenovirus 12 tumour antigen (Huebner et al. 1963), but no detectable CF antibody to purified adenovirus 12 hexon or fibre antigens. Purified hexon and fibre antigens were prepared from extracts of infected H.Ep–2 cells using step-wise elution from DEAE-cellulose columns (Huebner et al. 1964). Serum pools were also prepared from hamsters
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Preparation of T Antigens. Confluent monolayer cultures of HEK or H.Ep-2 cells were infected with adenovirus 12 at a multiplicity of 5 to 10 TCD50/cell. Virus was adsorbed for 2 hr at 37°C, the cells washed with two changes of Hanks’s saline and incubated for 72 hr in maintenance medium. After incubation, the cells were washed with two changes of 0.01 M-tris buffer containing 0.15 M-NaCl, pH 8.0 (0.01 M-tris-saline), and then scraped from the glass. The cells were suspended in 0.01 M-tris-saline, centrifuged at 200 g for 10 min., the cell pellet resuspended in 0.01 M-tris-saline to a concentration of 20% (v/v) and frozen at −80°C. Prior to serological tests and density-gradient studies, the cell suspensions were thawed, centrifuged at 20,000 g for 30 min. and the pellet discarded. These cell extracts contained T antigen (CF titre, 1:16 to 1:32) together with adenovirus 12 hexon and fibre antigens.

Preparation of tumour antigens. Tumour antigen extracts were prepared from monolayer cultures of both H212 and T.AdIII cell lines. Confluent cultures were washed with two changes of Hanks’s saline, scraped from the culture vessels and extracts prepared from 20% (v/v) suspensions of cells in 0.01 M-tris-saline as described for the preparation of adenovirus 12 T antigen extracts.

Antigen extracts were also prepared from CBA mice bearing transplanted adenovirus 12 tumours (Berman, 1967). A tumour-bearing CBA mouse was kindly supplied by Dr L. Berman from the National Institute for Medical Research, Mill Hill, London, and the tumours have been serially transplanted in vivo in our laboratory at 3- to 4-week intervals during the past 2 years. Subcutaneous tumours, approximately 10-15 mm. in diameter, were excised, washed with Hanks’ saline, minced with scalpels and centrifuged at 1800 g for 20 min. The pellet was suspended in 0.01 M-tris-saline to a concentration of 20% (v/v), briefly homogenized in a Waring blender and frozen at −80°C. Before further tests, the tumour extract was thawed, centrifuged at 20,000 g for 30 min. and the cell debris discarded. Tumour antigen extracts were also prepared from hamster tumours originating from the T.AdIII cell line of adenovirus 12 induced tumour cells. Hamsters, aged 3 to 4 weeks, were inoculated subcutaneously with 106 viable T.AdIII tumour cells cultured in vitro. Fragments of the tumours found 2–3 weeks later were inoculated into new-born hamsters (aged < 48 hr). Tumours approximately 10 mm. in diameter were removed 7 to 10 days after inoculation (Gilden, Beddow & Huebner, 1967), and antigen extracts prepared using the same procedures as described for the preparation of tumour antigen from transplanted adenovirus 12 tumours in CBA mice.

Rate-zonal centrifugation. Linear sucrose gradients were prepared with the aid of a mixing device from 5% and 20% sucrose solutions (Analar) in 0.01 M-tris-saline. Sucrose gradients of approximately 50 ml. volume were layered with 0.25 ml. of antigen extract containing either aldolase (200 µg.) or horse alcohol dehydrogenase (150 µg.); the enzymes were included to provide a marker of known molecular weight. After centrifugation at 100,000 g in a Spinco L preparative ultracentrifuge at 4°C, and using an SW–39 swinging-bucket rotor, 14 drop fractions of approximately 0.2 ml. were collected by bottom puncture. Each fraction was assayed for antigen and enzyme, and the molecular weight of virus antigens was estimated from their gradient positions compared with those of the enzymes (Martin & Ames, 1961). Where aldolase was used as a molecular-weight marker in rate-zonal centrifugation studies, the enzyme was

detected in three distinct peaks corresponding to the natural trimer, molecular weight $15 \times 10^4$ daltons, and the dimeric and monomeric forms. The molecular weights of virus-induced antigens were estimated with reference to the position of all three forms of the enzyme. Figure 1 shows an estimation of the molecular weights of the two species of adenovirus 12 T antigen (see Results). The position of the three molecular forms of aldolase were plotted against their molecular weights to give a standard line. The molecular weights of the antigen were estimated using the standard line and their observed position in the gradient.

**Fig. 1.** Estimation of molecular weight of adenovirus 12 induced T antigens. Peak positions of aldolase (○—○) and adenovirus 12 T antigens (●—●) in fractions from a 5% to 20% linear sucrose gradient layered with a sample of 0.25 ml. extract from adenovirus 12 infected H.Ep-2 cells and containing 200 µg. aldolase. Gradient centrifuged at 100,000 g for 15 hr.

**Complement fixation tests (CF).** These tests were carried out using overnight fixation at 4°C with two exact units of complement (Sever, 1962). The CF activity was expressed in Figures and Tables as the reciprocal of the highest antigen dilution which gave 3+ fixation against four standard units of antiserum.

In some tests for the detection of antigen in fractions from sucrose gradients, a modification of the CF technique of Wasserman & Levine (1961) was used. The titration of complement was carried out in the presence of 4 to 8 units of adenovirus 12 tumour hamster serum; the titre of this serum had been established previously by chessboard titration against adenovirus 12 tumour antigen using the method of Sever (1962). In tests for antigen, 0.25 ml. of antigen was incubated with equal volumes of serum, as used in the titration of complement, and 1:2 (50%) units of complement. Complement fixation was expressed as the difference in the spectrophotometer readings of the serum control and the test.

**Enzymes.** Aldolase was obtained as an ammonium sulphate precipitate (Worthington Biochemicals Ltd., Freetown, New Jersey). The precipitate was dialysed overnight.
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against 0.01 M-tris-saline before use. Aldolase was assayed by the method of Sibley & Lehninger (1949). Horse alcohol dehydrogenase (B.D.H., Poole, Dorset) was assayed by the methods of Theorell & Bonnichsen (1951).

RESULTS

Adenovirus 12 induced antigens in infected cell and tumour extracts

The specificity of the rabbit antisera to purified adenovirus 12 hexon and fibre antigens and of the adenovirus 12 tumour hamster serum is shown in Table 1. These sera, and other tumour hamster sera, were used in CF tests (Table 2) of adenovirus 12 induced antigens extracted from infected H.Ep-2 and HEK tissue cultures, and of tumour antigen extracts from transplanted adenovirus 12 tumours of hamsters and CBA mice and the H212 and T.AdIII tumour cell lines. Adenovirus 12 hexon, fibre and T antigens were present in antigen extracts from infected HEK and H.Ep-2 cells, but hexon and fibre antigens were not found in any of the four tumour antigen preparations.

Table 1. Activity of complement-fixing antibody in specific immune antiserum

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Adenovirus 12 hexon</th>
<th>Adenovirus 12 fibre</th>
<th>Adenovirus 12 tumour</th>
<th>Normal rabbit</th>
<th>Normal hamster</th>
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</thead>
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<tr>
<td>Adenovirus 12 hexon</td>
<td>160</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>Adenovirus 12 fibre</td>
<td>&lt; 4</td>
<td>320</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
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<tr>
<td>Adenovirus 12 hamster</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
<td>128</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>Normal H.Ep-2</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
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<tr>
<td>Normal hamster tissue</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
</tr>
</tbody>
</table>

* Reciprocal of serum dilution tested against four complement-fixing units of antigen

Table 2. Activity of complement-fixing antigen in virus-induced T and tumour extracts

<table>
<thead>
<tr>
<th>Antigen extract tested</th>
<th>Tissue</th>
<th>Adenovirus 12 hexon</th>
<th>Adenovirus 12 fibre</th>
<th>Adenovirus 12 tumour</th>
<th>sv40 tumour hamster</th>
<th>CELO tumour hamster</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus 12 T antigen</td>
<td>H.Ep-2</td>
<td>≥ 256</td>
<td>64</td>
<td>32</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
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<tr>
<td></td>
<td>HEK</td>
<td>≥ 256</td>
<td>64</td>
<td>16</td>
<td>N.T.</td>
<td>N.T.</td>
</tr>
<tr>
<td>Adenovirus 12 tumour antigen</td>
<td>CBA mouse tumour</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
<td>256</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
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<tr>
<td></td>
<td>H212 cells</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
<td>128</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
</tr>
<tr>
<td></td>
<td>T.AdIII cells</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
<td>16</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>sv40 tumour antigen</td>
<td>Hamster tumour</td>
<td>N.T.</td>
<td>N.T.</td>
<td>&lt; 4</td>
<td>64</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>CELO tumour antigen</td>
<td>Hamster tumour</td>
<td>N.T.</td>
<td>N.T.</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
<td>32</td>
</tr>
</tbody>
</table>

* Reciprocal of dilution of antigen tested against four units of serum. N.T. = not tested.
tested. Extracts from the H212 cell line and from transplanted CBA tumours showed relatively higher CF activity of tumour antigen than was found in T.AdIII cells or in transplanted tumours established from these cells. These differences were consistently obtained with a number of antigen extracts from each source. Adenovirus 12 T and

![Graph](image1)

**Fig. 2.** Rate-zonal centrifugation of adenovirus 12 hexon antigen. A sample of 0.25 ml. of antigen extract from adenovirus 12 infected H.Ep-2 cells, containing 200 μg. aldolase, was layered on to a 5% to 20% linear source gradient and centrifuged at 100,000 g for 4 hr. Fractions, collected by bottom puncture, were assayed for hexon antigen (●—●) and aldolase (○—○).

![Graph](image2)

**Fig. 3.** Rate-zonal centrifugation of adenovirus 12 fibre antigen. A sample of 0.25 ml. of extract from adenovirus 12 infected H.Ep-2 cells, containing 200 μg. aldolase, was layered on to a 5% to 20% linear sucrose gradient and centrifuged at 100,000 g for 12 hr. Fractions were assayed for fibre antigen (●—●) and aldolase (○—○).

tumour antigen preparations did not react with sera from hamsters bearing transplanted CELO virus or SV40 virus induced tumours, but these sera contained demonstrable CF antibody for homologous tumour antigen preparations.
Rate-zonal centrifugation studies of adenovirus 12 hexon and fibre antigens

Figure 2 shows the result of a rate-zonal centrifugation in a 5% to 20% linear sucrose gradient of an antigen extract from adenovirus 12 infected H.Ep-2 cells. Aldolase was included as a molecular-weight marker. After centrifugation at 100,000 g for 4 hr, fractions collected by bottom puncture were each assayed for aldolase and hexon CF antigen (Sever, 1962). The hexon antigen was detected as a single peak of CF activity indicating a molecular weight of 22 to 25 x 10^4 daltons.

Figure 3 shows the results of rate-zonal centrifugation studies of the adenovirus 12 fibre antigen. Fractions were collected from 5% to 20% linear sucrose gradients layered with antigen extracts from adenovirus 12 infected H.Ep-2 cells after centrifugation at 100,000 g for 12 hr. The sedimentation pattern of the fibre antigen was assayed using monotypic rabbit antiserum. Much of the fibre antigen was detected towards the bottom of the gradient, but a distinct band of antigen was detected in a position corresponding to a molecular weight of 6 to 7 x 10^4 daltons. The significance of the fibre antigen deposited towards the bottom of the gradient is not known.

Adenovirus 12 induced T antigen. Antigen extracts from adenovirus 12 infected H.Ep-2 cells and primary HEK cells were subjected to rate-zonal centrifugation in 5% to 20% linear sucrose gradients. After centrifugation at 100,000 g for 12 hr, fractions collected by bottom puncture were tested for aldolase and for T antigen, using adenovirus 12 tumour hamster serum. In the detection of this antigen complement fixation tests by the modified method of Wasserman & Levine (1961) were 5- to 10-fold more sensitive than the method of Sever (1962). Two peaks of T antigen CF activity were detected (Fig. 4), corresponding to molecular weights of 4 to 5 x 10^4 and 8 to 9 x 10^4 daltons. The presence of two molecular forms of T antigen was established in several rate-zonal centrifugation tests of extracts of adenovirus 12 infected H.Ep-2 and HEK cells.
Fractions obtained after rate-zonal centrifugation studies of extracts of adenovirus 12 infected H.Ep-2 cells were tested for hexon, fibre and T antigen. Separation of hexon antigen from T and fibre antigens was obtained in these tests. However, although the peak activities of fibre antigen and the two species of T antigen were

Fig. 5. Rate-zonal centrifugation of adenovirus 12 induced tumour antigen from transplanted CBA mouse tumours. A sample of 0.25 ml. of adenovirus 12 tumour antigen, diluted to a CF activity of 128 and containing 150 μg. horse alcohol dehydrogenase, was layered on to a 5% to 20% linear sucrose gradient and centrifuged at 100,000 g for 12 hr. Fractions were tested for tumour antigen (••) and alcohol dehydrogenase (○○).

Fig. 6. Rate-zonal centrifugation of adenovirus 12 induced tumour antigen from transplanted CBA mouse tumours. A sample of 0.25 ml. of adenovirus 12 induced tumour antigen, diluted to a CF activity of 32 and containing 200 μg. of aldolase, was layered on to a 5% to 20% linear sucrose gradient and centrifuged at 100,000 g for 12 hr. Fractions were assayed for tumour antigen (Wasserman & Levine, 1961; ••) and aldolase (○○).
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usually distinguishable, separation of these three antigens from each other was not found. Thus, the apparent similarity of molecular weights of the fibre antigen and, in particular, the larger species of T antigen, suggested that purification of these three antigens by rate-zonal centrifugation may not be practicable.

Adenovirus 12 induced tumour antigen. Adenovirus 12 induced tumour antigen extracted from H 212 and T.AdIII cells, and from transplanted tumours of hamsters and CBA mice, were each subjected to rate-zonal centrifugation. After centrifugation at 100,000 g for 12 hr, fractions were assayed for CF antigen, using the methods of Sever (1962). Horse alcohol dehydrogenase was included in the antigen extract as a molecular weight marker. A single peak of CF antigen activity was found (Fig. 5) in the same fraction as the peak activity of alcohol dehydrogenase (molecular weight 8.4 × 10^4 daltons; Ehrenberg & Dalziel, 1958).

In some cases, however, the presence of a smaller species of tumour antigen was suggested by the presence of a shoulder of CF activity. The presence of two tumour antigen species is shown in Fig. 6. Rate-zonal centrifugation studies were carried out on tumour antigen extracts, diluted to a CF activity of 16 to 32 and assayed by the method of Wasserman & Levine (1961). Most of the CF activity was detectable in a zone corresponding to an estimated molecular weight of 8 to 9 × 10^4 daltons; the minor species was estimated to have a molecular weight of 4 to 5 × 10^4 daltons. Similar results were found for all four tumour antigen extracts tested. Thus, tumour and T antigen extracts contained two species or molecular forms of antigen at the same indicated molecular weights. For the detection of the two species of tumour antigen, the CF activity of the sample was found to be important; the smaller species of tumour antigen was not distinguished in tests of tumour antigens of CF activity greater than 32 or less than 16.

In further tests, tumour antigen extracts were treated with RNase (150 μg./ml.) or with sodium deoxycholate (2.5 μg./ml.) for 2 hr at 30° prior to rate-zonal centrifugation. The sedimentation pattern of the tumour antigens was not affected by those treatments.

DISCUSSION

Rate-zonal centrifugation studies of antigen extracts from adenovirus 12 infected HEK and H.Ep-2 cells identified two species or molecular forms of T antigen of molecular weights 8 to 9 × 10^4 and 4 to 5 × 10^4 daltons. The larger antigen species is comparable to the larger of two antigen species identified by Hollingshead et al. (1967) and to the largest of four antigen species separated by Tockstein et al. (1968) (Table 3). The smaller of the two T antigen species found is comparable to the single species reported by Gilden (1968). Tockstein et al. (1968), reported two distinct T antigen species banding at a position corresponding to an estimated molecular weight of 4 to 5 × 10^4 daltons; in the present study, antigen in this position appeared to be a single species. An antigen species of estimated molecular weight 2.0 to 2.5 × 10^4 daltons was reported as the only T antigen species by Gilead & Ginsberg (1968), and as the smaller of two species by Hollingshead et al. (1967). These latter estimates of the molecular weight of the smaller antigen species do not necessarily conflict with the findings of Gilden (1968), and of the present study. Molecular-weight calculations based on rate-zonal centrifugation are estimates only and may be affected by experimental conditions. However, Tockstein et al. (1968) reported separate T antigen species of estima-
ted molecular weights $4 \text{ to } 5 \times 10^4$ and $2.2 \times 10^4$ daltons. The smaller species represented less than 1% of the total CF activity and may have been undetected in preparations of low activity.

The interpretation of molecular weights from sedimentation coefficients, as described by Martin & Ames (1961), assumes that molecules have the same shape and partial specific volume. As these molecular properties are not known for adenovirus 12 T and tumour antigens, the separation of multiple species of these antigens by rate-zonal centrifugation may indicate differences in molecular form rather than differences in molecular weight.

<table>
<thead>
<tr>
<th>Author</th>
<th>Source of antigen</th>
<th>Method of identification</th>
<th>No. species identified</th>
<th>Molecular weight $\times 10^{-4}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gilead &amp; Ginsberg (1968)</td>
<td>KB cells (+5FUdR)</td>
<td>Rate-zonal centrifugation</td>
<td>1</td>
<td>2.0-2.5</td>
</tr>
<tr>
<td>Hollingshead et al. (1967)</td>
<td>KB cells</td>
<td>Sephadex chromatography</td>
<td>2</td>
<td>8-9</td>
</tr>
<tr>
<td>Tockstein et al. (1968)</td>
<td>KB cells</td>
<td>Rate-zonal centrifugation</td>
<td>4</td>
<td>4.0</td>
</tr>
<tr>
<td>Present study</td>
<td>Various</td>
<td>Rate-zonal centrifugation</td>
<td>2</td>
<td>8-9</td>
</tr>
</tbody>
</table>

Rate-zonal centrifugation studies of adenovirus 12 induced tumour antigen initially indicated the presence of a single antigen species of estimated molecular weight 8 to $9 \times 10^4$ daltons in all four tumour cell extract studies. However, rate-zonal centrifugation studies of adenovirus 12 induced tumour antigen extracts indicated that two separable species of tumour antigen were present only if the CF assay of Wasserman & Levine (1961) was employed. The inability to demonstrate the two species of tumour antigen using the 5- to 10-fold less sensitive technique of Sever (1962) was attributed to a masking of the smaller antigens by a greater quantity of the larger species.

Although the two T antigen and two tumour antigen forms induced by adenovirus 12 appear to be similar, quantitative differences were evident. The larger antigen represented a greater proportion of the CF activity of the total tumour antigen than of the T antigen extracts. Similarity in the number and molecular weights of T and tumour antigens does not allow the assumption that the antigens are identical in the different extracts. Comparisons of the properties of adenovirus 12 induced T and tumour antigen in terms of other physical characteristics are required. Since treatment of tumour antigen extracts with RNase did not affect the sedimentation properties of the antigens they do not appear to be bound to RNA as described for sv40 virus induced tumour antigen (Gilden et al. 1965; Delvillano et al. 1968). However, it cannot be assumed that the antigen is not bound to RNA as some ribonucleoproteins are resistant to treatment with RNase. The sedimentation properties of adenovirus 12 induced tumour antigens were not altered by treatment with sodium deoxycholate, which suggested that the larger antigen was not an aggregate form of the smaller. Alterna-
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tively, the larger antigen may be an aggregate form which is not split by sodium deoxycholate.

Rate-zonal centrifugation studies of adenovirus 12 hexon and fibre antigen showed that these antigens were distinguishable from adenovirus 12 induced T antigens. Contamination of the larger T antigen by fibre antigen may account for some of the differences in serological activity of the T antigen species (Hollingshead et al. 1967). The estimate of $6 \times 10^4$ daltons for the molecular weight of adenovirus 12 fibre antigen is consistent with estimates of Hollingshead et al. (1967) and Maizel et al. (1968). Similarly, the estimates of 22 to $25 \times 10^4$ daltons for the molecular weight of the hexon antigen is within the range 20 to $40 \times 10^4$ daltons reported by others and reviewed by Maizel et al. (1968).

Further studies of the nature and function of adenovirus 12 T and tumour antigen may depend on purification of these proteins. As the form of these antigens appears to be independent of the cells in which they occur, extracts from transplanted tumours of CBA mice, which consistently provide antigen of relatively high CF activity, and which can be attained in large amounts, would provide suitable material for purification studies.

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