Antigenic Characterization of a Heterotransplanted Human Tumour, GW-127

Human neoplasms xenografted to laboratory animals for long periods of time have hitherto shown retainment of human antigen[1-4] and human chromosomes[3,5-8]. In terms of chromosomal constitution, the GW-127 tumour system, a human ovarian carcinoma successfully propagated in the cheek pouch of unconditioned golden hamsters (*Mesocricetus auratus*) in our laboratory (DMG) since August, 1966, is a striking exception, for it appears to resemble the hamster karyotype more than the human one[9]. It is thus of interest to examine the antigenic character of this tumour, particularly since, as the first of its kind, it regularly produces widespread metastases in its hamster host[10].

The species identification of this tumour was undertaken by means of the OUCHTERLONY two-dimensional agar gel immunodiffusion test[11,12]. Two GW-127 tumours of the 21st and 23rd passages were removed from their cheek pouches in January, 1967 (i.e. after a sojourn in hamsters for a little more than 5 months), homogenized 4 times for 3 sec (20,000 r.p.m.) with an Ultraturrax (Janke & Kunkel), centrifuged (Christ refrigerated centrifuge) at 4 °C and 15,000 r.p.m. for 30 min, and the supernatant concentrated to a total protein content of 1.5 to 2.0 g%. 1 ml of this extract was then added to 0.2 ml of antihamster serum and incubated at 37 °C for 2 h, and thereafter stored overnight at 4 °C. The latter procedure had to be repeated a second time in order to absorb all unspecific hamster components. The final extract was obtained as the supernatant resulting after centrifuging at 5000 r.p.m. for 10 min (4 °C). For purposes of comparison, the human adenocarcinoma (H.Ad. No. 1) heterotransplanted by Toolan to conditioned rats in 1955[13] and maintained by us in unconditioned hamsters since the end of 1964 was also included in our diffusion plates. GW-127 and H.Ad. No. 1 tumour extracts were tested against commercially available (Behringwerke, Marburg) antihuman and antihamster sera.

Figure 1 presents the precipitation results obtained prior to absorption with antihamster serum. It can be seen that GW-127 tumour extract shows about 4–5 precipitation lines against antihamster serum (AGS) and one definite line against antihuman serum (AHS). After complete absorption with AGS, this antihuman precipitation line is still present (Figure 2). To be sure, no qualitative difference between these 2 tumours can be documented at this time.

These findings indicate that whereas H.Ad. No. 1 has been shown to retain the human karyotype[3,7], while current evidence suggests that GW-127 more closely resembles that of the hamster[9], both do appear identically human in their antigenic constitution.

The presence of human antigen in a tumour also containing chromosomes from the host animal certainly invites speculation. Although one must question whether this human ovarian carcinoma's capacity to survive serial passage in a foreign, unconditioned host might be a result of viral oncogenesis, in which case the karyotype of the transplanted tumour would resemble the genotype of the host, we have proposed elsewhere[14] that the GW-127 tumour cells might have a mixture of chromosomes derived from both the tumour and the host. Indeed, such cases of interspecies hybridization have already been described in tissue culture systems[15-17]. The metastatic

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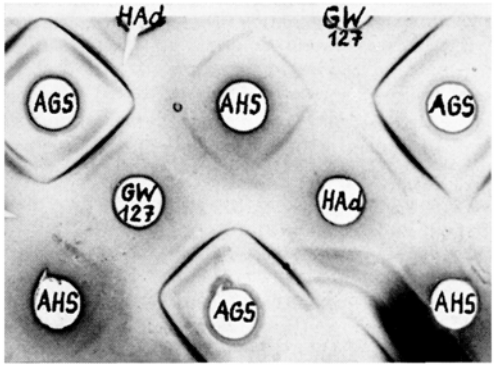

Fig. 1. OUCHTERLONY agar gel diffusion plate for human tumours GW-127 and H.Ad. No. 1 (AGS, antihamster serum, AHS, antihuman serum) before absorption with AGS.

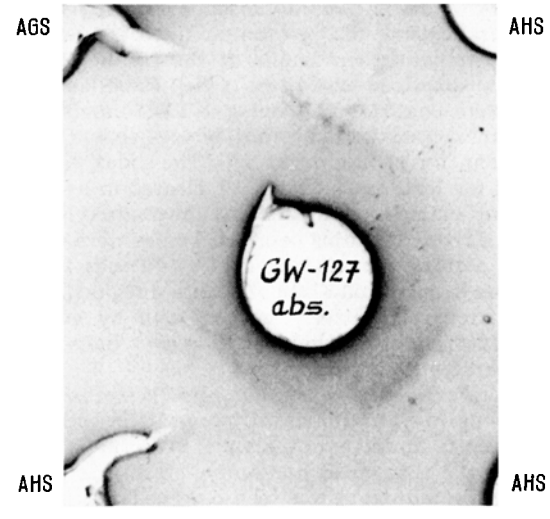

Fig. 2. Diffusion plate after complete absorption of GW-127 showing 1 precipitation line against antihuman serum and none against antihamster serum.

and invasive behaviour of this transplantable tumour of human origin makes the study of such questions imperative.

Zusammenfassung. Zweidimensionale Immunodiffusionsteste nach OUCHTERLONY zeigten, dass das im Hamster überpflanzte menschliche Ovarialkarzinom, GW-127, nach über 5 Monaten Tierpassagen noch humanes Antigen beibehalten hat. Die Möglichkeit einer Hybridisation von Humantumor- mit normalen Hamsterchromosomen wird zur Diskussion gestellt.

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Autoradiographic Localization of Testosterone-^{3}H in the Female Rat Brain and Estradiol-^{3}H in the Male Rat Brain

It is well known that male sex hormones trigger sex behavior in males, and female hormones trigger sex behavior in females of many species, including rats[1]. Sex hormones also affect sex behavior when injected into rats of the opposite sex. Testosterone injections increase the frequency of masculine mating responses and alter the strength of feminine mating behavior in ovariectomized or normal female rats[2]. Complementary effects are seen when estrogens are injected into castrated male rats[3].

As part of the analysis of sex hormone effects on reproductive functions, testosterone uptake in the brain of the male rat has been described[4,5], as has estradiol uptake in the female brain[6–9]. The present report describes testosterone-^{3}H uptake in the female rat brain and estradiol-17β-^{3}H uptake in the male rat brain.

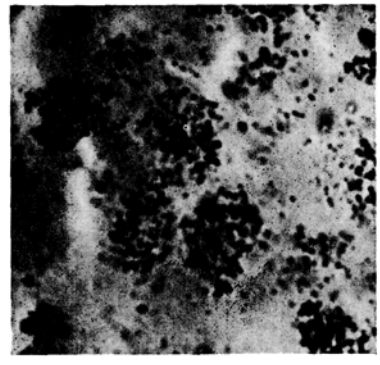
Testosterone-1,2-^{3}H (200 μc; sp. act. 46.5 c/mM; New England Nuclear Corp.) was injected i.v., dissolved in 0.05 cm^3 25% ethanol, into each of two 200 g female rats, ovariectomized 2 weeks previously. Estradiol-17β-6,7-^{3}H (200 μc; sp. act. 42.4 c/mM; New England Nuclear Corp.) was injected i.v., dissolved in 0.05 cm^3 25% ethanol, into each of two 200 g male rats, castrated 2 weeks previously. 2 rats were killed $^1/_2$ h after injection (1 of each sex) and 2 rats were killed 2 h after injection (1 of each sex). The brain of an uninjected male rat was prepared in the same way as the experimental brains, to check for the presence of autoradiographic artifacts.

The brains were frozen quickly after removal, and 8 μ serial frozen sections were cut in a cryostat, mounted directly onto slides and rapidly dried. Then the tissue was fixed by immersion in 1% osmium tetroxide and 10% neutral formalin. Other details of the histological procedure are described elsewhere[9]. The fixed and dried sections were coated with Kodak NTB-3 emulsion, redried and exposed in lightproof boxes packed with a drying agent, for 11 months at 5 °C. The slides were then developed for 7 min in Kodak D19, cleared in hypo, and some were stained with Mayer's haematoxylin. The numbers of reduced grains over cell bodies were counted in 22 brain regions, including about 100 cells for each brain region in each animal. In other brain regions, uptake was estimated qualitatively, rather than by counting. Care was taken to insure that sampling from brain regions was comparable for all animals.

Radioactive hormone was taken up in cells throughout the female brain $^1/_2$ h after testosterone-^{3}H injection (e.g. Figure). Most limbic-hypothalamic structures showed greater uptake than most non-limbic structures (Table). No such grain reduction was seen over cells in the brain of the uninjected control animal.

In most regions of the female brain, testosterone-^{3}H uptake was much lower 2 h after injection than it was $^1/_2$ h after injection (Table). However, in certain anterior limbic structures – the preoptic area, prepiriform cortex, olfactory tubercle, septum and olfactory bulb granule cells – and in the cingulate gyrus, uptake after 2 h remained high. Prolonged uptake of this sort is characteristic of steroid hormone target structures[10].

Radioactivity was detected in cells throughout the male brain $^1/_2$ h after estradiol-17β-^{3}H injection. Uptake tended to be higher in limbic-hypothalamic structures than in non-limbic structures, but it was not uniformly so (Table). Estradiol-^{3}H uptake after 2 h was high in the preoptic area, prepiriform cortex, olfactory tubercle, septum and cingulate gyrus, a pattern similar to testosterone-^{3}H distribution in the female brain. However, in contrast to the testosterone-^{3}H distribution, estradiol-^{3}H



Autoradiograph showing labeling of 5 cells in the lateral preoptic area of a female rat injected with testosterone-^{3}H. The section is unstained. The clumps of reduced grains are known to be located over cell bodies, from examination of adjacent, stained autoradiograms. × 1200.

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