The data provided by the 2 experiments are given separately in the Table, which clearly reveals a positive result. The homogeneity between the two sets of data is remarkable, as also the similar response to all 3 insulin doses. The number of cell lines obtained is $^{13}/_{120}$, i.e. 10.83%. The only cell line obtained withlout insulin (i.e. $^{1}/_{40}$ or 2.5%) corresponds to expectation, since it conforms to the results obtained by the authors already quoted. At the present date (end of October 1975), 9 lines are on their way to becoming established, i.e. to reach the conventional number of passages (70). 4 further lines are in an earlier stage. A preliminary survey to determine the caryotype of the lines obtained indicates that all of them are predominantly diploid. The individual chromosomes look normal or close to normality.

As a general conclusion it seems that insulin increases significantly continuous growth in primary cultures of embryonic cells of *Drosophila*. The action mechanism of insulin remains to be ascertained, as also the optimal dose. The lack of difference between the results of the 3 doses employed may signify that all were far above the minimum required for influencing cell growth.

Nuclear Projections in Tumour Cells and Large Chromosome Markers

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Summary. The application of banding techniques on cytological smears from pleural effusion in a case of histiocytic sarcoma has provided direct evidence for correspondence between nuclear projections in tumor cells and extra large chromosome markers observed in the neoplastic karyotype obtained by direct preparations.

Several authors[1-4] have reported the presence of nuclear projections in the interphase nuclei of various tumours in association with large chromosome markers. These protrusions are readily observed in well flattened neoplastic cells of the chromosome preparations as well as in cytological smears, histological sections[5], and even in blocks prepared from neoplastic pleural effusions[6].The size of the chromosome markers and the nuclear volume appear to influence significantly the observation of the projections: in fact abnormally long chromosomes and diploid or hypodiploid sets seem to favour the detection of these nuclear extrusions[6].

In this connection we should like to add direct evidence that the nuclear projections do in fact correspond to the presence of extremely long chromosome markers.

Direct chromosome preparations from a pleural effusion in a patient affected by histiocytic sarcoma showed in 94% of the metaphases the presence of a pseudodiploid karyotype characterized by a giant submetacentric chromosome (Figure 1) whose length was approximately 2,5 times the length of the long arm of the A_1 chromosome. Banding techniques with quinacrine[7] and trypsin[8] demonstrated that this abnormal chromosome was constituted by a whole A_1 chromosome joined with a large

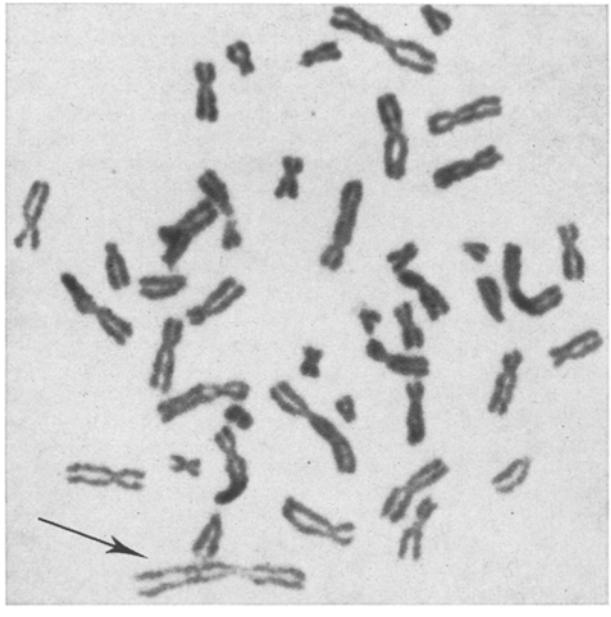

Fig. 1. Pseudodiploid metaphase showing a giant submetacentric chromosome marker (arrow). Direct preparation from pleural effusion.

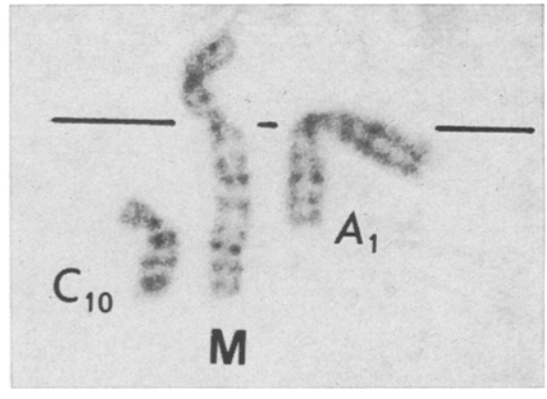

Fig. 2. Structural constitution of the marker chromosome demonstrating the involvement of a whole A_1 chromosome and a large segment of the q arm of a C_{10} chromosome (trypsin banding technique).

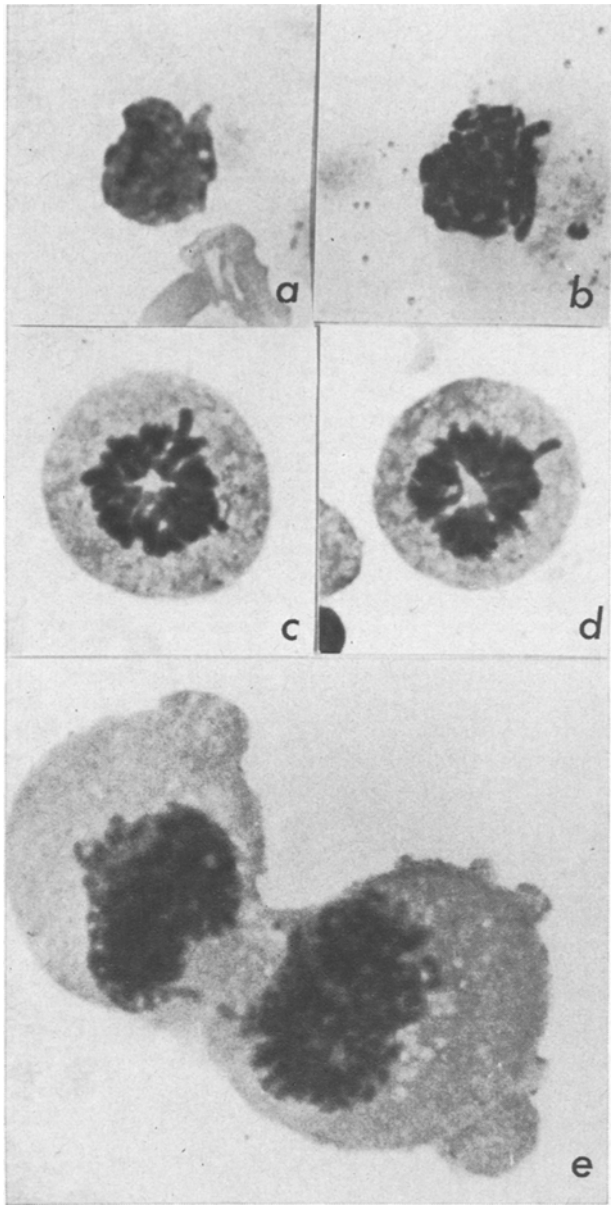

Fig. 3. Different features of an interphasic nucleux and spontaneous mitotic figures showing the protrusion of single projections: a) interphasic nucleus; b) prophase; c, d) metaphases; e) anaphasic picture demonstrating the presence of the giant marker as an elongated chromosome protruding from the masses of the migrated chromosomes. May-Grünwald – Giemsa; a, b, c, d) ×1,300; e) ×2,400.

segment almost corresponding to the long arm of a C_{10} chromosome (Figure 2). Since the cytological examination of the pleural sediment in our patient showed single prominent nuclear projections in most of the cells and of the spontaneous mitoses (Figure 3), these preparations were treated with the conventional G banding techniques in order to identify directly on the cytological smears the nature of the extruded chromosome arms. Best results were obtained by employing trypsin digestion[8] for a rather long period of incubation (up to 30 min).

In our case 72% of the spontaneous metaphases showed, after trypsin treatment, that the extruded chromosome segments were actually constituted by the terminal portions of the marker chromosome observed in the suitably spreaded metaphases (Figures 4). No preferential pattern of extrusion of the projections was observed, since both proximal and distal segment of the marker chromosome were equally distributed in the banded spontaneous mitoses.

These results suggest that the detection of a very high number of mitoses with extruded segments, corresponding to extra-large chromosomes, is an indirect confirmation of the fact that abnormal markers apparently encounter a normal division during the mitotic process, but that their organization in the interphasic nuclei may still require further elucidation.

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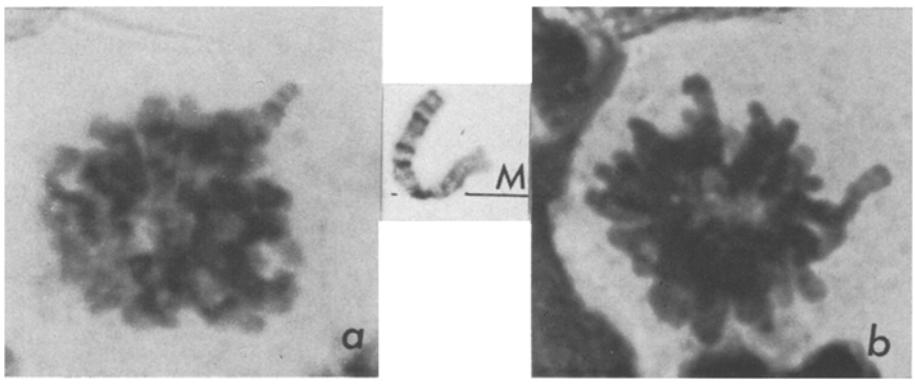

Fig. 4. Spontaneous mitoses in cytological preparations treated with trypsin and demostrating the extrusion of the arms of a chromosome with the same banding pattern of the proximal (b) or distal (a) portion of the marker chromosome (M).