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Growth of Human Epidermoid Carcinomas (Strains KB and HeLa) in Hamsters from Tissue Culture Inocula.* (22224)

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Eagle has reported the isolation of a human epidermoid carcinoma (strain KB) directly from a biopsy specimen(1) in a new medium which satisfies the specific amino acid and vitamin requirements of a mouse fibroblast (strain L) and the HeLa strain of epidermoid carcinoma(2-5). Since strain KB was isolated directly in these culture media without the intervening use of experimental animals, the purpose of the present report is to record its successful transplant from *in vitro* cultures to the cheek pouches of Syrian hamsters. Similar experiments with the HeLa cell also were undertaken, since this tissue culture strain apparently has not yet been studied in the hamster cheek pouch.

Methods. Strain KB, isolated Dec. 21, 1954 and maintained by serial subculture in these new media(1), and strain HeLa(6), similarly maintained in these media for more than a year, were obtained through the courtesy of Dr. Harry Eagle, National Institutes of Health. Both strains had been cultivated in these laboratories for some 4-6 months prior to the present experiments by regular subculture in the medium described by Eagle (2-5), which contains 13 essential amino acids, 7 essential vitamins, glucose and salts, each in the concentration necessary for maximal growth of these 2 cell lines(1-5). The essential serum protein was provided in the present experiments by 10% whole, fresh human serum. The layer of cells growing on the surface of the culture flasks was removed for subculture and for animal experiments by replacing the culture fluid in 8-10-day-old cultures with fresh media containing Difco "1:250" trypsin in a final concentration of 0.125%, and incubating the flask *circa* 5 min at 37°C. The dispersed cells were then sedimented at 5-600 rpm, washed 2 or 3 times with 10-15 ml of culture media to remove the trypsin and resuspended in a convenient volume of complete culture media. The suspensions were shaken gently to disperse the cells, and after enumeration by direct haemocytometer counts, diluted so that the desired number of cells was contained in a volume of 0.1 ml of media. Syrian hamsters, 60-80 g in weight, were prepared for implantation as described by Lutz *et al.*(7). The 0.1 ml of culture media containing the desired inoculum was implanted under the epithelium of the cheek pouch with a 24 gauge needle. Cortisone acetate in subcutaneous doses of 2-3 mg was administered simultaneously with implantation and twice weekly post-implantation(8).

Results. Strain KB. The implantation of 16,000 cells produced a visible nodule in the cheek pouch (Fig. 1a) following a latent period of 56 days. The nodules continued to grow and eventually filled the pouch, presenting the gross appearance of a typical cheek pouch tumor (Fig. 1b). The histology of these tumors† is consistent with that of an

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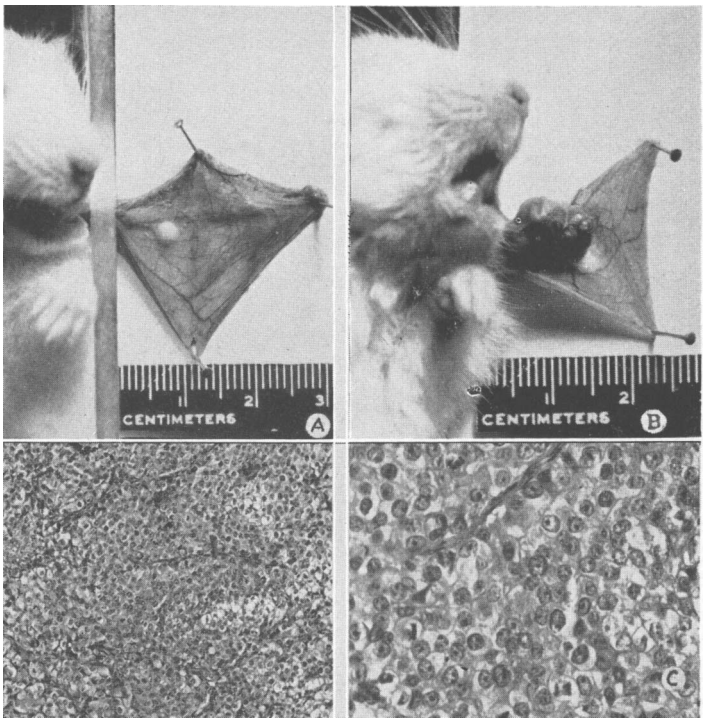

FIG. 1. Implantation of human epidermoid carcinoma, strain KB in hamster cheek pouch from tissue culture inocula. a. Implantation of 16,000 cells, 56 days. b. Same tumor, 76 days. c. Histologic section, 76 days. H. & E., 140 × and 440 ×.

epidermoid carcinoma (Fig. 1c). The latent period following implantation from *in vitro* culture has decreased progressively with successive hamster passages until now after the 12th passage, it must be transplanted every 10 days. The tumor grows in about 80% of the animals implanted, and if not transplanted on schedule, ulceration develops and the tumor becomes necrotic between 12-15 days. The animals then expire, usually as the result of secondary infection. Preliminary dose-response experiments done in duplicate with bilateral implantation in each animal indicate that the implantation of *circa* 15-20 cells results in the development of a tumor following a latent period of 153 days. Samples of tumor from the 9th passage of strain KB in cortisonized hamsters were implanted successfully in the cheek pouches of untreated animals. This tumor line now has been transplanted through 3 successive passages in non-conditioned hamsters. The tumor growth rate thus far is slower than in cortisone-treated animals. These tumors if not transplanted, undergo ulceration and necrosis between 22 and 25 days.

Tissue culture inocula of strain KB also have been implanted subcutaneously into LAF_1 mice bearing intramuscular transplants of the homologous ACTH secreting pituitary tumor reported by Furth *et al.*(9) according to the method described by Handler(10). Tumors developed in 80% of the mice bearing the pituitary tumor, attaining volumes of approximately 1 ml in thirty days. All mice died of the effects of the pituitary tumor. Thus far, strain KB has failed to grow in normal mice.

Strain HeLa. The implantation of 18,600 cells produced a nodule in the cheek pouch (Fig. 2a) following a latent period of 14 days. These nodules developed the gross appearance of typical cheek pouch tumors (Fig. 2b), the histology† of which is consistent with that of an epidermoid carcinoma (Fig. 2c). The latent period following implantation from *in vitro* culture has decreased to 10 days after 4 successive hamster passages. The tumor

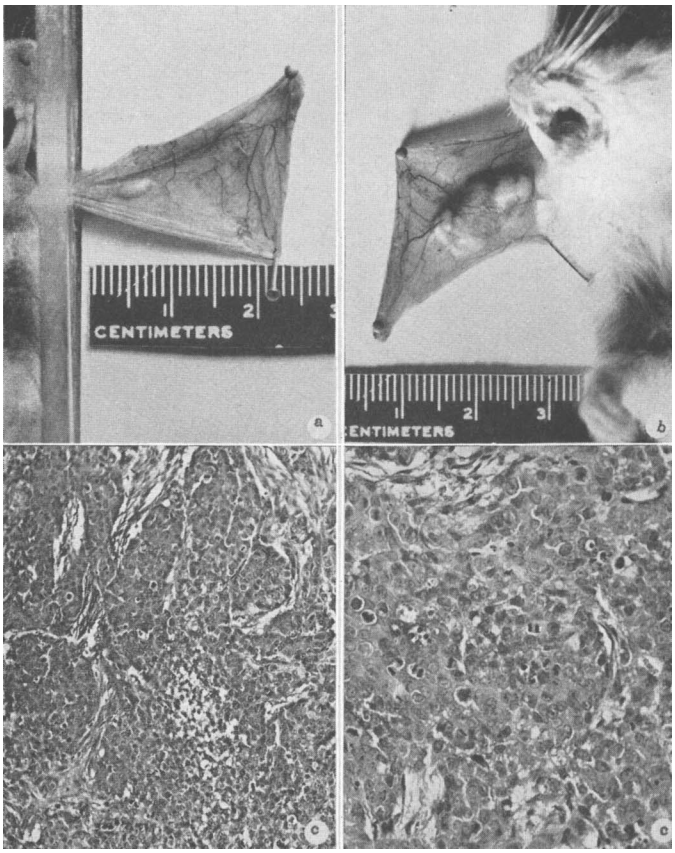

FIG. 2. Implantation of human epidermoid carcinoma, strain HeLa in hamster cheek pouch from tissue culture inocula. a. Implantation of 18,600 cells, 14 days. b. Same tumor, 34 days. c. Histologic section, 34 days. H. & E., 75 × and 220 ×.

grows in 60% of the animals implanted, and unless transplanted every 18 days, the cheek pouch ulcerates, the tumor becomes necrotic between 20-24 days and the animals die of secondary infection. Preliminary dose-response experiments done in duplicate with bilateral implantation in each animal indicate that the implantation of *circa* 15-20 cells results in the development of a tumor following a latent period of 92 days.

Discussion. The present experiments with strain KB represent the first instance in which a human tumor isolated directly from a biopsy specimen in these new media has been transplanted from cultures to experimental animals. Similarly, so far as is known, strain HeLa has not heretofore been implanted successfully in the hamster cheek pouch. These observations indicate a simple method by which various tumor cell lines isolated and/or maintained in these media can be checked periodically for tumor production *in vivo*, and further suggest the possibility that the same tumor cell lines may be established and used in parallel in these media and in the hamster cheek pouch for the comparative screening of potential chemotherapeutic agents.

The uniform tumors produced by measured tissue culture inocula should be particularly useful in the chemotherapeutic study of cheek pouch tumors. Quantitated cell suspensions prepared by the trypsinization of biopsy specimens(11) of certain other human tumors being carried in hamsters(12) have been equally effective when implanted into the hamster cheek pouch, as have been similar hamster and mouse tumor inocula when im-

planted appropriately in homologous hosts (12). These preliminary experiments also suggest that the latent period may be predetermined within reasonable limits by the number of tumor cells contained in the inoculum.

Summary. 1. The KB and HeLa strains of human epidermoid carcinoma have been established in the cheek pouches of golden hamsters by means of quantitated suspensions prepared from tissue cultures in a medium providing the specific amino acid and vitamin requirements of these cell lines. 2. Strain KB, isolated directly from a biopsy specimen in these media, has been grown in LAF_1 mice bearing homologous ACTH secreting pituitary tumors. This strain also has been established in the cheek pouch of non-conditioned hamsters but thus far has failed to grow in non-conditioned mice. 3. The use of quantitated tissue culture inocula for the production of tumors in the hamster cheek pouch for chemotherapeutic studies is suggested.

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Effects of Temperature and Work on Metabolism and Heat Loss in Man.*† (22225)

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Muscular exercise in a cold environment by either man(1) or animals(2,3) requires a greater oxygen consumption than the same activity in a warm environment. In man, the added energy requirement for work in the cold is not entirely the added hampering effect of clothing(4). Hart(2) concluded that the extent to which heat produced by activity is used to maintain body temperature depends upon the effect of activity on over-all physiological body insulation. The following experiments were devised to disclose changes of insulation in man exercising in warm and cool environments.

Methods. Skin temperature, heat loss and oxygen consumption were measured in 4 male subjects clad in shorts and exercising in cooling and cool environments. The experiments were begun at an ambient temperature of 20°C. The temperature was then steadily decreased to 10°C over ½ hour, and maintained at 10°C during the remainder of the test period (30 to 60 minutes). The subject reported shivering on a 4-point scale. Exercise on a bicycle ergometer was performed at a rate of 150 kg m/min or 70 kg m/min. Exercise was initiated with the start of cooling in some experiments, and after shivering had begun as a result of cooling in others. Skin temperature was measured with copper-constantan thermocouples recorded by a Brown

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