the presence of EPF is 7 percent as compared to 0.1 percent in the control cultures.

Thus, TI reflects the number of cells incorporating thymidine for cell division, not just DNA repair or movement of the cells into the DNA synthesis period of the cell cycle. Our in vitro assay system has several advantages over other bioassay procedures (*3, 6*) in that the specific response of one cell type is measured in the absence of other cell types, we can screen a large number of tumors and tumor cell lines for the production or content of EPF, and we require less tumor material.

The production of EPF in vitro by clonal cell lines, coupled with the in vitro assay, provides a system for the purification and characterization of EPF. Neither the EPF described by us nor the TAF described by Folkman has been adequately characterized. These factors may prove to be identical; however, we note that our factor differs from Folkman's (*4, 6*) in the following ways: (i) it is produced by tissue culture cells; (ii) it is ribonuclease resistant; (iii) it is sensitive to proteolytic digestion with Pronase; and (iv) it stimulates the proliferation of endothelial cells but not of fibroblasts. Cavallo (*7*) suggests that the nonspecificity of TAF may result from the lack of a purified factor; this may also contribute to the differences we observe between TAF as described in vivo and EPF produced in tissue culture. Neither TAF nor EPF appears to be species specific as demonstrated by the increased proliferative response of human endothelial cells elicited by tumor cells of both rodent and human origin. The in vivo effects of EPF have yet to be determined.

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References and Notes

1. G. H. Algire and H. W. Chalkley, *J. Natl. Cancer Inst.* **6**, 73 (1945).
2. I. F. Tannock and S. Hayashi, *Cancer Res.* **32**, 77 (1972).
3. M. A. Gimbrone, Jr., S. B. Leapman, R. S. Contran, J. Folkman, *J. Exp. Med.* **136**, 261 (1972).
4. J. Folkman, *Am. J. Med.* **285**, 1182 (1971).
5. H. P. Wright, *Nature (Lond.)* **220**, 78 (1968).
6. J. Folkman, E. Merler, C. Abernathy, G. Williams, *J. Exp. Med.* **133**, 275 (1971).
7. T. Cavallo, R. Sade, J. Folkman, R. Cotran, *Am. J. Pathol.* **70**, 345 (1973).
8. E. A. Jaffe, R. L. Nachman, C. G. Becker, C. R. Minick, *J. Clin. Invest.* **52**, 2745 (1973).
9. P. Benda, J. Lightbody, G. Sato, L. Levine, W. Sweet, *Science* **161**, 370 (1968).
10. G. Augusti-Tocco and G. Sato, *Proc. Natl. Acad. Sci. U.S.A.* **64**, 311 (1969).
11. J. J. Tumilowicz, W. W. Nichols, J. J. Cholon, A. E. Green, *Cancer Res.* **30**, 2110 (1970).
12. R. L. Suddith, P. J. Kelly, H. T. Hutchison, E. A. Murray, B. Haber, in preparation.
13. Supported by NIH grants NS 11354 and NS 11255, the Lanier Foundation, and Neurosurgery MSRDP research funds. We thank K. Werrbach for technical assistance and the medical and nursing staff of the Department of Obstetrics and Gynecology for providing the umbilical cords from patients at term.

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Production of Globules in Mouse L Cells Penetrated with Hamster Sperms

Abstract. *Globule formation has been observed in long-term cultures of mouse L cells penetrated with hamster spermatozoa. These numerous, uniform, and spherical structures are approximately 10 micrometers in diameter when formed, and are positive to stains specific for proteins and nucleic acids. They may be produced either by sperms within their target cells or by the cells in response to penetration by the spermatozoa.*

Active penetration of spermatozoa into somatic tissues was initially observed by Kohlbrugge (*1*) in the female genital epithelia of domestic fowls and mammals. It was further demonstrated in vitro by Reid (*2*), who incubated (in tissue culture) human sperms with biopsy materials from the postparturient uterus. Both intercellular and intracellular penetrations were evident upon examination of the tissues after short-term culture. Reid also noticed the formation of vacuoles and the alignment of mitochondria around the heads of sperms within their target cells, thus indicating some form of interaction between the host cells and the spermatozoa which penetrated them. Extensive works by Bendich *et al.* (*3*) have shown that, in tissue culture, mouse sperms are capable of penetrating various mammalian cell lines. Within their target cells, the release of labeled DNA from mouse spermatozoa, followed by the production of substances immunologically specific to the sperm donor, have also been demonstrated, implying that protein synthesis might have been initiated by the spermatozoa as a result of their entry.

I now report a cellular activity hitherto not observed, namely, the production of globules by a line of mouse fibroblasts penetrated with spermatozoa from a hamster.

In my experiment, motile spermatozoa, aseptically extracted from the cauda epididymis of a Syrian hamster, were mixed with mouse L cells in tissue culture medium (*4*) supplemented with 0.5 percent fructose to enhance sperm motility (*5*). There were ten sperms to each cell, and the final density in the mixture was approximately 5×10^5 cells per milliliter. Adhesion of sperm heads to cells was observed immediately after mixing, and vigorous vibration was maintained by the tails for 8 to 12 hours while the heads remained tightly bound to their target cells. As sperm motility subsided, the cells settled down and spread out on microscope slides that were incubated under standard tissue culture conditions at 34°C. Most cells were attached with single sperms, but multiple attachment was not infrequent (Fig. 1). Within 12 hours after admixture of sperms and L cells, intracellular location of sperm heads was evident; and in 48 hours most of these intracellular sperm heads had already disappeared in the cytoplasms of their target cells (Fig. 1). In agreement with observations by Bendich *et al.* (*3*), each target cell then began to display more than one nucleus, indicating the arrest of cell division due to sperm penetration. From then on, the cells increased both in size and in nuclear number until, in 8 to 12 days, a population of polynucleates prevailed in which most cells exhibited more than eight nuclei (Fig. 2). Due to the low density of plating and the large initial intercellular distances, it seemed quite unlikely that these giant cells could have arisen from the fusion of adjacent cells. Studies on their chromosomal numbers revealed a widespread polyploidy among these giant cells, suggesting endomitosis might have been the mode of cell growth subsequent to sperm penetration (*6*).

Further incubation of the sperm-penetrated cells gave rise to numerous spherical structures hitherto nonexistent in the culture (Figs. 3 to 5). And, depending on the state of the host cells before admixture with spermatozoa, the appearance of these globules came within 2 to 3 weeks after sperm entry. These uniform globules can be seen inside both giant polynucleates and smaller cells (Fig. 3). They are also visible around those cells from which they seem to have been extruded (Fig. 4), and they appear in aggregates on sites where giant cells may have disintegrated to yield a group of mononucleate cells together with the globules (Fig. 5). The formation of globules seemed to be a fairly synchronous activity, with a peak period of about 4 days during which most cells were associated with a number of these spherical objects.

The globules average about 10 μm in diameter when sparsely attached to glass.

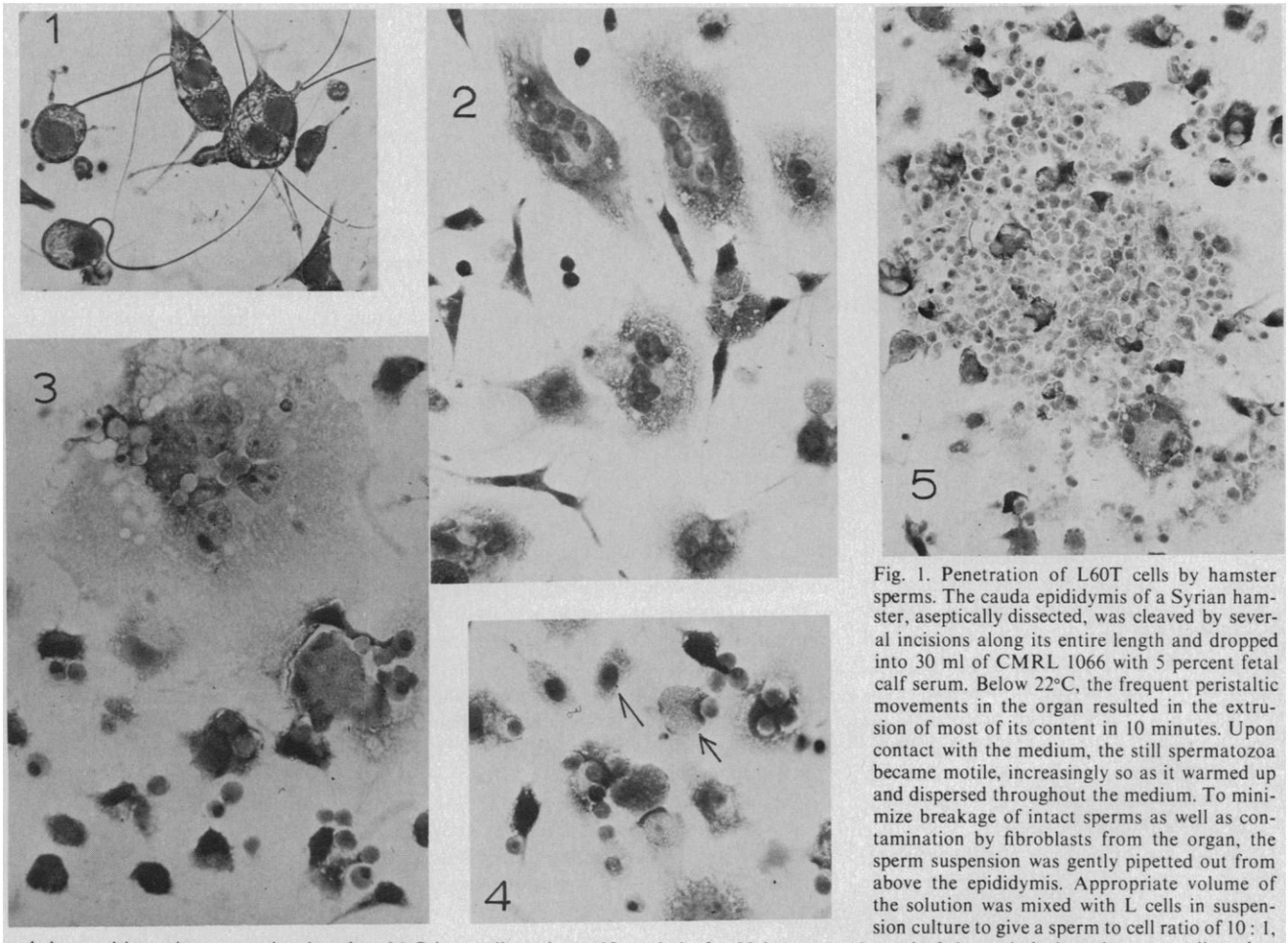

Fig. 1. Penetration of L60T cells by hamster sperms. The cauda epididymis of a Syrian hamster, aseptically dissected, was cleaved by several incisions along its entire length and dropped into 30 ml of CMRL 1066 with 5 percent fetal calf serum. Below 22°C, the frequent peristaltic movements in the organ resulted in the extrusion of most of its content in 10 minutes. Upon contact with the medium, the still spermatozoa became motile, increasingly so as it warmed up and dispersed throughout the medium. To minimize breakage of intact sperms as well as contamination by fibroblasts from the organ, the sperm suspension was gently pipetted out from above the epididymis. Appropriate volume of the solution was mixed with L cells in suspension culture to give a sperm to cell ratio of 10 : 1, and the resulting mixture was incubated at 34°C in a roller tube at 30 rev/min for 20 hours. At the end of the period, the culture was allowed to settle on glass bottle from which it was periodically scraped off, resuspended, and plated over cover slips in petri dishes. Photomicrograph shows cells 48 hours after admixture with hamster sperms. Preparation was air dried, Giemsa stained for 5 seconds, and cleared with tap water. No mounting resin has been used for this particular specimen in order to give a distinct image of the sperm tails by which the multiplicity of penetration may be assessed. Cells with double nuclei are common at this stage of incubation. Most intracellular sperm heads have by now disappeared within the cytoplasm, leaving behind some vacuole-like structures in their places. For more detailed studies of the earlier stages of sperm penetration, see Bendich *et al.* (*3*) (× 500). Fig. 2. Prevalence of giant cells as seen in a culture 9 days after admixture of L cells with hamster sperms. As the number of nuclei had multiplied within most cells, so had the cytoplasms become increasingly more granular and dark against Giemsa stain (× 300). Fig. 3. Appearance of globules in L cells 14 days after penetration by hamster sperms. Globules are seen to be forming within the giant cell on the upper half of the picture. On the lower half, they are at various stages of either being formed or separated from their host cells of different sizes. The densely stained structures borne by some globules have given them the appearance of cells in miniature (× 500). Fig. 4. Progression of globules away from their apparent place of origin. The line-up of the globules may serve as a clue as to the sequence of their manufacture as well as the direction of their migration from the host cell. Besides the globules, the appearance of the netlike expanded nucleus (←) and mononucleate without clear boundary (←) are both unique to the sperm-penetrated cells at this stage of development (× 500). Fig. 5. Liberation of globules by one or more giant cells in a culture 18 days after admixture of hamster sperms and L cells. The membrane of the polynucleate has disappeared and the outline of the cell is no longer discernible. The densely stained daughter cells of the dissolved giant polynucleate are crowded amid the globules and are much distorted (× 300).

They are positive to amido black and Feulgen stains, indicating, respectively, that proteins and nucleic acids form parts of their composition. Autoradiography showed their incorporation of tritiated thymidine and amino acids after labeling for short periods of these precursors to DNA and proteins (*6*). Globules of similar dimension and chemical makeup are also displayed by L cells after admixture with mouse spermatozoa.

The appearance of globules in somatic cells has been a reproducible event subsequent to sperm penetration. It has been observed, with similar recurrence, in cultures of HeLa, Chinese hamster ovary cells, and primary mouse and hamster kidney cells with either mouse or hamster epididymal sperms. However, it has not been observed in parallel control cultures in which sperm-free epididymal extracts were used for the admixture. Thus, barring unaccountable contamination and artifacts, these spherical structures may be regarded as substances produced either by sperms within their target cells, or by the host cells in response to penetration by spermatozoa.

Together with the generation of the multinucleated giant cells (Fig. 2), the production of globules seems to represent a full course of interaction between mouse L cells and hamster spermatozoa after their union at penetration. With the cessation of globule production came a population of predominantly spindle-shaped mononucleates which are morphologically different from the original mouse fibroblasts. Whether there are alterations in the genotype as well as phenotype of this culture is unknown.

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References and Notes

1. J. H. F. Kohlbrugge, *Arch. Entwicklungsmech. Org.* **35**, 165 (1913).
2. B. L. Reid, *Lancet* **1964-I**, 21 (1964).
3. A. Bendich, E. Borenfreund, S. S. Sternberg, *Science* **183**, 856 (1974).
4. Original characterization of L60T the target cell used in these experiments, is given → J. E. Till, G. F. Whitmore, S. Gulyas, *Biochim. Biophys. Acta* **72**, 277 (1963).
5. T. Mann, *The Biochemistry of Semen and of the Male Reproductive Tract* (Methuen, London, 1964), pp. 266–272.
6. L. C. Lau, in preparation.
7. Abstract was presented in part at the 19th Annual Meeting of the Biophysical Society and published in *Biophys. J.* **15** (2, Pt. 2) 58a (1975). Supported by the National Cancer Institute of Canada. I thank A. M. Rauth for criticism and the supply of various somatic cell lines used for the experiments.

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Preferences for Sweet and Salty in 9- to 15-Year-Old and Adult Humans

Abstract. *Preferences for the tastes of sucrose, lactose, and sodium chloride were measured in 618 subjects between 9 and 15 years of age and in 140 adults. The younger subjects preferred greater sweetness and saltiness than did the adults. In the younger group, there were race and sex differences in preferences, none of which appeared among the adults.*

Factors controlling the consumption of sugar and salt are of current general interest. Sucrose and sodium chloride are responsible for most of the sweetness and virtually all of the saltiness of the foods making up the current human diet. Individuals vary widely in their preferences for sweetness and saltiness (*1*). The individual's preferences persist over a relatively long period of time (*2*), which suggests that they are characteristic of the person rather than of his immediate metabolic state. These taste preferences may be the major factor mediating the amounts of sucrose and sodium chloride consumed.

In an earlier study, we found sex and race differences in preferences for sweet and salty among 9- to 15-year-old subjects (*2*). Now we find that those between 9 and 15 years of age differ from adults in their preferences for these two taste qualities.

A group of 140 adults (*3*) was tested in a manner identical to that used with the 9- to 15-year-old subjects in the earlier study (*2*). Each subject was given preference tests for sucrose, lactose, and sodium chloride, in that order. For each test, four cups containing different concentrations of the compound were presented. The concentrations were 0.075, 0.15, 0.30, and 0.60*M* sucrose; 0.10, 0.20, 0.30, and 0.40*M* lactose; and 0.05, 0.10, 0.20, and 0.40*M* sodium chloride (*4*). The subject tasted the four samples without swallowing them, and then ranked them in the order of preference from most to least preferred.

The percentage of subjects in each age group (*5*) selecting each concentration as their most preferred is given in Fig. 1. In the case of sucrose, approximately one-fourth of the adults selected each of the concentrations as his most preferred, whereas 50 percent of the children selected the most concentrated sample as the one they like the best ($\chi^2 = 27.1$, d.f. = 3, $P < .01$). The same pattern appeared with lactose ($\chi^2 = 12.0$, d.f. = 3, $P < .01$), although it was not so pronounced with this less sweet and less acceptable (*6*) sugar. This age difference in preference for sweetness correlates with recent U.S. Department of Agriculture estimates of sugar consumption: a 1965 survey indicates that the per capita consumption of sugar and sweets is greater among those 9 to 15 years of age than among adults (*7*).

The two age groups also differed in their salt preferences ($\chi^2 = 8.3$, d.f. = 3, $P < .05$). The largest percentages of both groups indicated that they most preferred the mildest salt solution, but among the younger subjects, there was a relatively large group that selected the saltiest sample. Thus, for all three of these compounds, more 9- to 15-year-old subjects than adults preferred the more concentrated solutions (*8*).

These first-choice preference data were analyzed further for effects of sex and race. Among those 9 to 15 years of age, males selected sweeter lactose solutions than did females ($\chi^2 = 8.5$, d.f. = 3, $P < .05$). Although the differences were not statistically significant, there were trends in this direction among the adults and among both age groups for sucrose. This observation also conforms to intake estimates in the United States: after age 9, males consume greater quantities of sweets than do females of the same age (*7*). No sex differences appeared in either group for salt preferences.

The distribution of first choices among the black and white subjects in the two age groups are given in Fig. 2. In the younger group, more of the black than the white subjects selected stronger concentrations of sucrose ($\chi^2 = 17.1$, d.f. = 3, $P < .01$), lactose ($\chi^2 = 10.6$, d.f. = 3, $P < .05$), and sodium chloride ($\chi^2 = 67.4$, d.f. = 3, $P < .01$) as their most preferred. None of

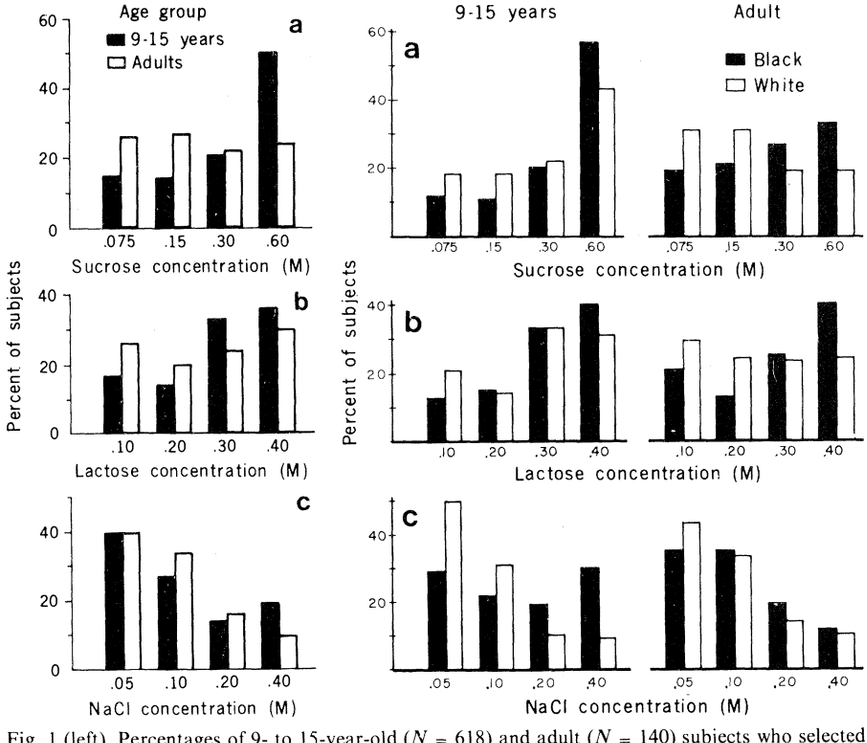

Fig. 1 (left). Percentages of 9- to 15-year-old ($N = 618$) and adult ($N = 140$) subjects who selected different concentrations of sucrose (a), lactose (b), and sodium chloride (c) as their most preferred concentration. Fig. 2 (right). Percentages of 9- to 15-year-old blacks ($N = 310$) and whites ($N = 308$) and adult blacks ($N = 52$) and whites ($N = 88$) who selected different concentrations of sucrose (a), lactose (b), and sodium chloride (c) as their most preferred concentration.