complement. Further, the cells consistantly fail to provoke tumors in appropriate hosts, and have been maintained for up to 20 months and 27 pasages in culture. These normal characteristics have been retained despite the fact that the only difference in treatment of these control cells from that of the transformed cells was the presence or absence of benzo(a)pyrene.

DIPAOLO et al.[8,9] performed chromosomal analyses of chemically transformed hamster and rat cultures and of tumors induced by these cells. They found that the cells were, generally, near-diploid. Analysis of our transformed cultures show chromosomal anomalies in 83% of the dividing cells, including 5 different types of aberrations. We are currently engaged in chromosomal analysis of other transformed hamster cell lines to determine if these aberrations are specific for B(a)P induced transformation or are common to other transformations in culture.

In the present work, the successful maintenance of normal characteristics in the control cell lines, grown for extended periods, may be due to the utilization of an improved Eagle's medium and extended time between passages. Both of these factors apparently facilitate adaptation of hamster embryo cells to culture conditions.

Summary. Two normal diploid control cell lines and a heteroploid malignant transformed cell line from B(a)P treated hamster embryo cell cultures were established. The 14-month-old B(a)P transformed cell line grew 8-times faster than the 20-month-old control cell line. The control cell line showed normal diploid chromosome complement in 93% cells and heteroploidy in 7% cells while B(a)P treated line showed 83% heteroploid cells and only 17% diploid cells. This is the first report on the establishment of diploid hamster cell cultures grown for extended period.

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[8] J. A. DIPAOLO, N. C. POPESCU and R. L. NELSON, Cancer Res. *33*, 3250 (1973).

[9] C. D. OLINICI and J. A. DIPAOLO, J. natn. Cancer Inst. *52*, 1627 (1974).

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[11] Acknowledgments. This work was supported by the French Ministry of Quality of Life, by the Institut National de la Santé et Recherche Médicale, by the S.E.I.T.A. and by Contract No. 12-14-7001-297 of the Agricultural Research Service, U.S. Dept. of Agriculture, Administered by the Athens, Georgia Area, Richard B. Russell Agricultural Research Center, Athens, Georgia 30604, USA. We thank Mr. B. R. SHARMA, Mr. E. J. RADIN, Dr. R. HAKIM and Dr. VED BRAT for their help during this work.

Potency of Thyroid Hormone Analogues in Suppressing Prolactin-Mediated Mammary Growth in Thyroidectomized Rats

It has been recently demonstrated that following thyroidectomy the mammary epithelium of the rat undergoes extensive growth due to an increased sensitivity to endogenous prolactin[1]. Administration of replacement doses of thyroxine to such animals prevents this exaggerated mammary growth response. A similar prolactin-thyroxine antagonism may be implicated in the pathogenesis of human beast cancer[2,3]. BERN et al.[4,5] and MITTRA[1] have proposed that prolactin-thyroxine interaction at the level of the mammary epithelium may be analogous to that known to occur in amphibian tissues at metamorphosis. It was therefore of interest to investigate the effect on mammary epithelium of two thyroid hormone analogues, 3,5,3′-triiodothyropropionic acid (TRIPROP) and 3,5,3′-triiodothyroacetic acid (TRIAC), which have relatively weak calorigenic and goitre prevention activities in the rat[6], while their potency in inducing tadpole metamorphosis is comparable to[7], or even greater than[8], that of the natural thyroid hormones thyroxine (T_4) and triiodothyronine (T_3).

Material and methods. Virgin, female Sprague-Dawley rats weighing about 200 g were used for the experiment and were fed on a commercial diet. All rats were given oestradiol-17β (in 50% propylene glycol) 8 μg s.c. daily from Day 1 to Day 10 and killed at the termination of the experiment on Day 16. Rats in Group I (Figure 1) received no further treatment and were allowed tap water *ad libitum*. The remainder were surgically thyroidectomized on Day 1 and, to ensure complete endogenous thyroid hormone deficiency, were concurrently started on 3-amino-1,2,4-triazole (0.1% solution) in the drinking water until completion of the experiment. This anti-thyroid drug has been shown to have little effect on the peripheral deiodination of T_4[9]. The thyroidectomized animals were divided into 9 groups (Group II–X, Figure 1). Group II received no other treatment while the remainder received variously T_4, T_3, TRIPROP and TRIAC at dose levels of 2.0 and 0.2 μg/100 g body wt., i.p. daily from Day 1 to Day 15 (Figure 1). At completion of the experiment the left thoracic mammary glands were excised and processed for whole mount preparation. The degree of mammary gland development was rated on the standards shown in Figure 2 by an independent observer. The mammary scores of rats in the various groups were compared with those of Group II using a one-way analysis of variance of normal scores[10].

[1] I. MITTRA, Nature, Lond. *248*, 525 (1974).

[2] I. MITTRA and J. L. HAYWARD, Lancet *1*, 885 (1974).

[3] I. MITTRA, J. L. HAYWARD and A. S. MCNEILLY, Lancet *1*, 889 (1974).

[4] H. A. BERN and C. S. NICOLL, Recent. Prog. Horm. Res. *24*, 681 (1968).

[5] D. V. SINGH and H. A. BERN, J. Endocr. *45*, 579 (1969).

[6] N. R. STASILLI, R. L. KROC and R. I. MELTZER, Endocrinology *64*, 62 (1959).

[7] E. FRIEDEN, Proc. 2nd Int. Congr. Endocr., London (Excerpta Medica Foundation, Amsterdam 1964), p. 35.

[8] J. C. KALTENBACH, exp. Zool. *174*, 55 (1970).

[9] L. VAN MIDDLESWORTH, S. L. JONES and C. E. CHAPMAN, Physiologist *3*, 169 (1960).

[10] M. G. KENDALL and A. STUART, in *The Advance Theory of Statistics* (Charles Griffin, London 1967), vol. 2, p. 465.

Results. As shown previously[1], in oestrogen treated rats thyroidectomy leads to marked stimulation of mammary growth (Figure 1). In the thyroidectomized animals administration of all the compounds tested inhibited this mammary stimulation at a dose of 2 μg/100 g body wt. This is within the accepted dose of T_4 required to maintain euthyroidism in thyroidectomized rats, and hence this dose of T_4 and also T_3, which is several times more active than T_4[6], were capable of preventing mammary growth in these animals. However, it is of interest that TRIPROP which has been reported to have only 10% of the metabolic potency of T_4, and 0.8% of T_3, and TRIAC 21% and 2.6% of the metabolic potencies of T_4 and T_3 respectively[6], were also capable of preventing mammary stimulation in the thyroidectomized rats (Figure 1). Moreover, when $^1/_{10}$ of the above dose was used (0.2 μg/100 g), TRIPROP still had an effect comparable to that of T_3 (Figure 1), although this dose of TRIPROP could have had little effect on oxygen consumption. At this lower dose, however, neither TRIAC nor T_4 had any significant suppressive effect on mammary growth.

Discussion. The present experiment demonstrates that TRIPROP and TRIAC, despite their relatively weak calorigenic and goitre-prevention activities, have an inhibitory effect on mammary growth which is comparable to that of T_3 and T_4. This preferential effect of the two analogues on the mammary gland would appear to be due to a selective affinity of these compounds for mammary epithelial cells, compared with that for other tissues primarily responsible for mediation of calorigenic action. However, no information regarding the potency of TRIPROP and TRIAC in specifically stimulating oxygen consumption of mammary cells is presently available. In any event, this 'anti-growth' effect of TRIPROP and TRIAC on mammary epithelium would be mediated via peripheral blockade of prolactin action, similar to that demonstrated for thyroxine[1,5,11].

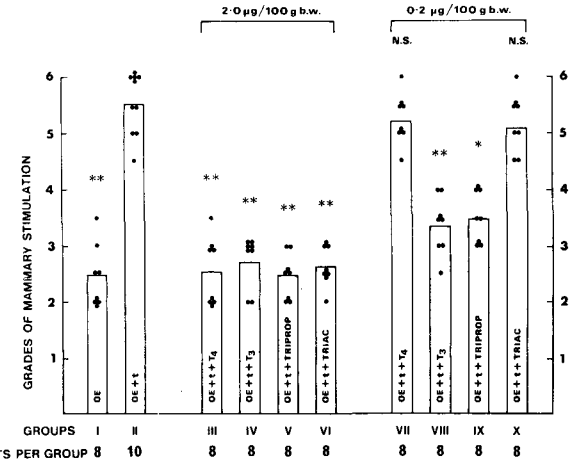

Fig. 1. Histogram showing the suppressive effect on thyroidectomy-induced mammary stimulation of T_4, T_3, TRIPROP and TRIAC in the rat. The columns represent mean mammary score in each group; the dots, mammary score of individual rats. All rats received oestradiol (OE) daily from Day 1 to Day 10 and were killed on Day 16. Groups II–X were thyroidectomized (t) on Day 1 and maintained on the anti-thyroid drug Aminotriazole until Day 16. For dosage of oestradiol and Aminotriazole see text. The various thyro-active compounds were given daily in a dose of 2.0 μg/100 body wt. to groups III–VI and 0.2 μg/100 g body wt. to groups VII–X. The compounds were dissolved in a minimum volume of 0.1 *N* NaOH, diluted to attain the required concentration and neutralized with 0.1 *N* HCl. The final volume injected per rat was 0.2 ml. **indicates $p < 0.01$ and *indicates $p < 0.05$ significant difference in mammary grading compared with group II.

The induction of tadpole metamorphosis by thyroid hormones is considered to represent a change in genetic expression, although there is no convincing evidence to suggest a direct interaction between thyroid hormones and genes responsible for this transition[12]. Prolactin on the other hand can prevent certain of the metamorphic changes in the amphibia, particularly tail resorption[13–15], by antagonizing thyroxine at the peripheral tissue level, and is considered to be a hormone responsible for stimulation of growth of larval structures[4]. One may speculate that prolactin while stimulating larval growth acts as an obligatory repressor of certain of the genes that determine metamorphosis to an adult frog, and that thyroid hormones, which reach an optimal concentration at metamorphosis[14], bring about their expression essentially by antagonizing prolactin at the tissue level, thereby indirectly activating genetic expression. The situation would then by analogous to that in insects[13,14] where metamorphosis of larvae is precipitated by the 'turning off' of juvenile-hormone which is assumed to occur in the presence of a high titer of ecdyson[16]. The present experiment demonstrates that in addition to the natural thyroid hormones certain of their analogues, despite a weak calorigenic effect, are equally capable of antagonizing prolactin. Perhaps it is this anti-prolactin rather than thyromimetic property of the two analogues studied, and possibly of other structurally related compounds[8,17,18] which is responsible for their relatively high potency in inducing tadpole metamorphosis, especially when measured in terms of tail regression[8,18].

Prolactin is an essential factor for promotion of growth in carcinogen-induced hormone-dependent mammary tumours in the rat, and experimental reduction of prolactin secretion results in regression of such tumours[19,20]. Abolition of prolactin secretion may cause regression of human breast cancer[20]. Treatment with thyroactive substances, on the other hand, is thought to have little effect on the progression of mammary cancer[21,22]. But,

[11] J. Meites and C. L. Kragt, Endocrinology *75*, 561 (1964).
[12] J. R. Tata, in *Biochemical Actions of Hormones* (Ed. G. Litwack; (Academic Press, New York 1970), vol. 1, p. 89.
[13] H. A. Bern, C. S. Nicoll and R. C. Strohman, Proc. Soc. exp. Biol. Med. *126*, 518 (1967).
[14] W. Etkin, in *Metamorphosis* (Eds. W. Etkin and L. I. Gilbert; Appleton-Century Crofts, New York 1968), p. 313.
[15] R. C. Jaffé and I. I. Geschwind, Proc. Soc. exp. Biol. Med. *146*, 961 (1974).
[16] L. I. Gilbert, Recent. Prog. Horm. Res. *30*, 347 (1974).
[17] W. L. Money, S. Kumaoka, R. W. Rawson and R. L. Kroc, Ann. N.Y. Acad. Sci. *86*, 512 (1960).
[18] R. J. Lindsay, L. Buettner, N. Wimberly and J. A. Pittman, Gen. comp. Endocr. *9*, 416 (1967).
[19] J. Meites, J. natn. Cancer Inst. *48*, 1217 (1972).
[20] O. H. Pearson, A. Molina, T. P. Butler, L. Llerena, and H. Nasr, in *Oestrogen Target Tissue and Neoplasia* (Ed. T. L. Dao; Univ. Chicago Press, Chicago 1972), p. 287.
[21] J. Hayward, in *Recent Results in Cancer Research* (Springer-Verlag, Berlin 1970), vol. 24.
[22] R .H. Wilkins and D. L. Morton, Cancer *16*, 558 (1963).

this belief is based on studies on transplanted mouse mammary tumours[22] that become essentially hormone independent after induction[23], or with the use of only physiological doses in the rat[24] and man[25-27]. JULL and HUGGINS[28], however, reported that thyroxine given in supraphysiological levels drastically inhibits the growth of carcinogen-induced mammary tumours in the Sprague-Dawley rat. Similarly, oestrogen, which inhibits mammary tumour growth by blocking the peripheral action of prolactin[19, 29], is only effective when given in pharmacological doses[19]. It may, therefore, be fruitful to study the effect of large doses of a suitable thyroid hormone analogue, with divergent calorigenic and mammary anti-prolactin effects, on experimental and human mammary cancer, perhaps in conjuction with an agent that will, additionally, reduce pituitary prolactin secretion.

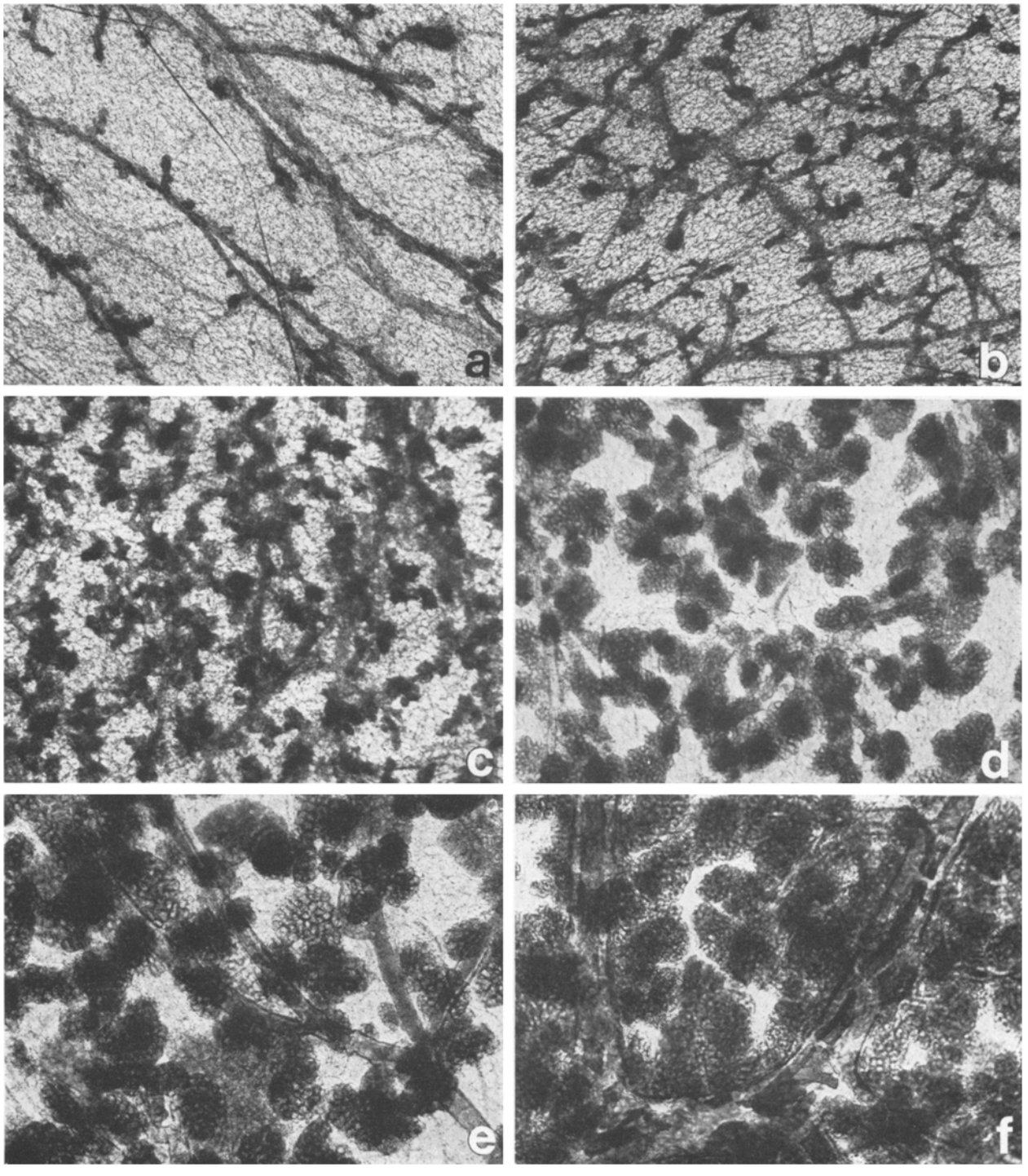

Fig. 2. Representative photographs of whole-mount preparations of rat mammary gland (× 50) depicting various grades of development and stimulation. a) Grade 1, a few ducts and end buds. b) Grade 2, moderate number of ducts and end buds. c) Grade 3, numerous ducts and end buds, no lobulo-alveolar development. d) Grade 4, minimal lobulo-alveolar development. e) Grade 5, moderate lobulo-alveolar development. f) Grade 6, marked lobulo-alveolar development.

Summary. An antagonism between prolactin and thyroxine, similar to that found in amphibian tissues at at metamorphosis, has been recently shown to occur at the level of the rat mammary epithelium. This phenomenon may be implicated in the pathogenesis of human breast cancer. This experiment demonstrates that two analogues of thyroid hormone, triiodothyropropionic acid and triiodothyroacetic acid, which are relatively very weak in their calorigenic action, are as potent as thyroxine and triiodothyronine in inhibiting the prolactin-mediated mammary growth in thyroidectomized rats. The possible implication of this finding in the treatment of mammary cancer is discussed.

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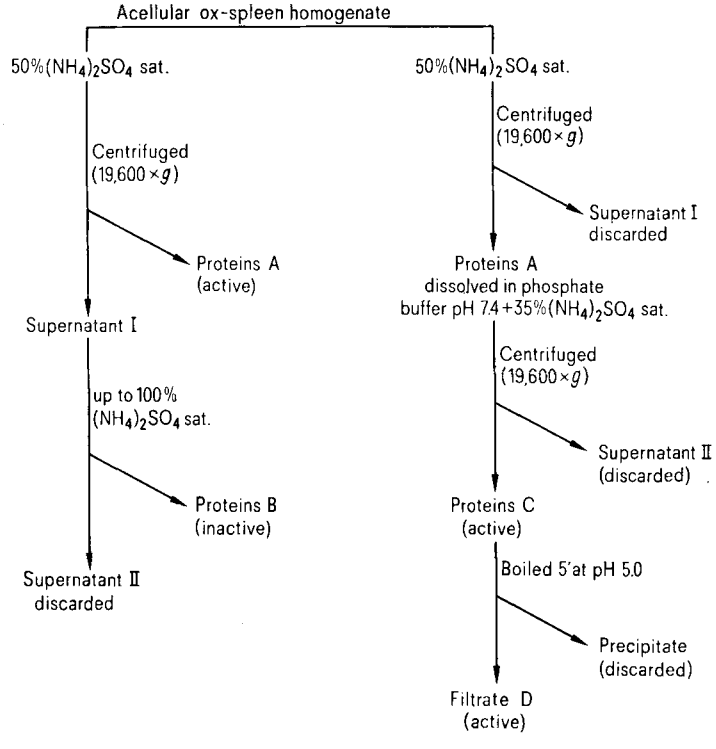
Imperial Cancer Research Fund, Breast Cancer Unit, Guy's Hospital, London, SE1 9RT (England), 16 May 1975.

[23] O. MUHLBOCK, J. natn. Cancer Inst. *48*, 1213 (1972).
[24] W. C. NEWMAN and R. C. MOON, Cancer Res. *28*, 864 (1968).
[25] E. W. EMERY and W. R. TROTTER, Lancet *1*, 358 (1963).
[26] A. R. LYONS and G. A. EDELSTYN, Br. J. Cancer *19*, 116 (1965).
[27] R. M. O'BRYAN, G. S. GORDAN, R. M. KELLEY, R. G. RADVIN, A. SEGALOFF and S. G. TAYLOR, Cancer *33*, 1082 (1974).
[28] J. W. JULL and C. HUGGINS, Nature, Lond. *188*, 73 (1960).
[29] C. W. WELSCH and E. M. RIVERA, Proc. Soc. exp. Biol. Med. *139* 623 (1972).
[30] I am much indebted to Dr. J. R. TATA of National Institute of Medical Research, London and Professor H. A. BERN of University of California at Berkeley for helpful criticism of the manuscript.

Preliminary Purification and Dosages of the Erythropoietic Factor from Ox-Spleen

The existence of a splenic erythropoietic stimulating factor is well known; in fact, since 1926, KRUMBHAAR[1] emphasized the indirect influence of the spleen on blood formation through a stimulating action on bone marrow. Later GLEY, DELOR and LAUR and RUHENSTROTH-BAUER[2] concluded that the spleen may represent one of the sites producing an erythropoietic-stimulating factor. More recently DE FRANCISCIS[3,4] reported the results of his investigations: Acellular ox spleen homogenate, when injected i.p. in rats, caused a great increase of reticulocytes in peripheric blood. In mice there was no increase of reticulocyte concentration after treatment with homogenates of kidney, muscle or liver of splenectomized animals; there was, on the contrary, a pronounced and significant reticulocyte increase in the animals treated with spleen extract or with liver of normal rats.

Table I.



In this work we proposed to study a purification method for this active factor and to assay its erythropoietic activity with different methods.

Materials and methods. An acellular ox-spleen homogenate was prepared by homogenization of the organ, immediately removed after the animals death, with 2 volumes of distilled water in a blender at 15,000 rpm for 2 min and at 0 °C. The coarse homogenate was then centrifuged for 90 min at 54,000 × *g* and at 0 °C. The acellular supernatant was precipitated by different percentages of ammonium sulphate following the reported outline (Table I).

[1] E. B. KRUMBHAAR, Phys. Rev. *6*, 160 (1926).
[2] G. RUHENSTROTH-BAUER, Arch. exp. Path. Pharmak. *32*, 211 (1950).
[3] P. DE FRANCISCIS, G. DE BELLA, B. MASTURSI and S. CIFALDI, Boll. Soc. ital. Biol. sper. *39*, 1779 (1963).
[4] P. DE FRANCISCIS, DE BELLA G. and S. CIFALDI, Science *150*, 1831 (1965).