Summary

Addition of a tiny crystal of boric acid to a drop of buffered water containing male and female gametes of *Euallomyces javanicus* completely inhibits copulation in this water mold.

Pure gametophytic growth can thus be secured by addition of 1/15,000 boric acid to the starch-yeast extract medium.

The Experimental Induction of Pseudogamy in Early Mouse Embryos

Pre-fertilisation treatments which eliminate the sperm or the egg chromosomes have long been known to produce gynogenetic or androgenetic development respectively in Amphibian embryos[1]. Treatment of the sperm is of two kinds: by irradiation[1] using radium, ultra-violet, or X-rays; or by chemicals[2], e.g. trypaflavine and toluidine blue[3]. Few attempts to reproduce the Amphibian results in the Mammalia have as yet been made. Ultra-violet irradiation of rabbit sperm by PINCUS and ENZMANN[4] and by BEATTY and SELMAN (unpublished), and X-irradiation of similar material by AMOROSO and PARKES[5], severely affected fertilisation and early cleavage. Both PINCUS and ENZMANN[4] and AMOROSO and PARKES[5] inferred the induction of gynogenesis from their histological examination of the fertilized eggs. In addition, haploid blastocysts are known to occur in the mouse both spontaneously in stocks giving a high incidence of heteroploid (chiefly triploid) embryos[6], and also following colchicine treatment at fertilisation designed to induce triploids[7].

Experiments have been carried out using the mouse (*Mus musculus*) to investigate further the possibilities of causing haploid gynogenetic development in a mammal. Treatment of the mouse sperm was made on the lines previously successful in the Amphibia[8]: two being chemical treatments using trypaflavine and toluidine blue, and two irradiations by ultra-violet or X-rays. Three and a half days after artificial insemination with treated sperm, the developing embryos and the unfertilised eggs were washed from the uterus of the inseminated females. The embryos were squashed and stained, and chromosome counts made (to an accuracy of $\pm$ 5% of the total chromosomes) on the mitotic figures found. Typically the untreated mouse embryo is a blastocyst at three and a half days development.

Irradiation of the sperm by ultra-violet or X-rays produced a series of abnormal embryos. Three criteria were used to judge these embryos: their stage of development as shown by their nuclear number, their chromosome count, and their cytological appearance. With both irradiations nuclear number was inversely correlated with dosage up to the highest used (1·6 Joules per cm^2 between wavelengths 2100–3200 A°, and 50,000r respectively on the surface of the sperm suspension), though the X-rays gave a more uniform effect than the ultra-violet. The high dosages restricted development to the first cleavage division. Chromosomally, the X-rays produced a series of embryos which showed a gradual reduction from the diploid (i.e. 40) to the haploid complement with increasing dosage, this reduction being probably the result of an increasing inactivation of the sperm chromosomes by the X-rays. The ultra-violet, on the other hand, produced two different kinds of development over a wide range of dosages, one being diploid or near-diploid and the other haploid or near-haploid; a result which suggested an all-or-none response to the ultra-violet by the sperm chromatin. Most of the near-diploids were hypo-diploid by a few chromosomes. Cytologically, in addition to apparently normal nuclei, many embryos contained very small nuclei which, together with evidence of contracted and lagging chromatids, indicated mitotic irregularities during development. These cytological defects were probably the result of damage to the division centre or chromosomes of the sperm by the irradiations, and may be the cause of the hypo-diploid embryos obtained from the ultra-violet.

Trypaflavine treatment of the sperm at first suggested a HERTWIG effect[1], for at a concentration of 1/10,000 one haploid blastocyst and one triploid embryo were obtained among several diploids. But the haploid, taken from a female possibly heterozygous for the silver factor, could have been of spontaneous origin[2]. With both trypaflavine and toluidine blue two distinct kinds of development were induced; one kind was similar to controls in the stage of development reached, the other was highly retarded. All the classifiable normal embryos were diploid with the exception of the haploid and triploid mentioned previously.

The haploid and near-haploid embryos obtained from the irradiations were presumably gynogenetic in origin. All had low cell numbers when compared to controls, and were developing with difficulty. The retarded embryos from the chemical series were similar to those produced by the high irradiation dosages and by analogy with them are probably also gynogenetic. It is evident that haploid gynogenetic development in the mouse produced by these treatments is qualitatively different from that induced in the Amphibia[3] where gynogones often develop to metamorphosis; and as the results given here are closely comparable to the previous results from the rabbit[4], it appears that haploid gynogenesis in the Mammalia is highly abnormal and probably confined to the early cleavage stages.

In contrast to the retarded appearance of the haploids obtained from the irradiation series, the single haploid blastocyst obtained from the trypaflavine treatment, the haploids obtained from the colchicine treatment[5], and the spontaneous haploids[6] all appeared healthy and had cell numbers closely comparable to their diploid sibs. The contrast between the two types of haploid is even more marked when the other classes of heteroploid embryos accompanying the haploids are examined. The trypaflavine, colchicine, and spontaneous haploids occur often with triploids, whereas no triploids occur in the irradia-

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tion series. This duality can be resolved if, by an effect on the maturation spindle, the healthy haploids are androgenetic, and have arisen through the whole of the female chromosome complement entering the second polar body at fertilization, leaving only the sperm nucleus within the egg. A similar mass retention of all the maternal chromosomes in the egg is the most likely cause of triploid development, consequently the relation between the trypaflavine, colchicine, and spontaneous haploids and triploids can be explained by these mechanisms. If this hypothesis is correct, the haploid androgenetic development of mouse embryos is more successful than, and is more similar to its counterpart in the Amphibia[1] than is the haploid gynogenetic development obtained by the irradiations.

These results will be given elsewhere in detail.

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Zusammenfassung

Nach Behandlung von Mäusesperma mit ultraviolettem Licht oder Röntgenstrahlen wurden bei vielen dreieinhalb Tage alten Embryonen von der Norm abweichende Chromosomensätze gefunden. Verschiedene waren haploid oder nahezu haploid; diese vermutlich durch Gynogenese entstandenen Embryonen waren gewöhnlich in ihrer Entwicklung zurückgeblieben. Behandlung der Spermatozoen mit Trypaflavin oder Toluidinblau führte zu ähnlich verzögerter Entwicklung, aber ohne klar beobachtbare Abnormalitäten des Chromosomensatzes. Es wird eine Theorie der gynogenetischen und androgenetischen Entwicklung von Mausembryonen aufgestellt, und und mit ähnlichen Entwicklungstypen bei Amphibien verglichen.

[1] G. FANKHAUSER, Quart. Rev. Biol. *20*, 20 (1945).

Action de la ribonucléase sur les cellules du carcinome d'Ehrlich

On sait que, d'après l'hypothèse de BRACHET[1], les acides ribonucléiques joueraient un rôle important dans la synthèse des protéines, la division cellulaire et le développement embryonnaire. Cette hypothèse nous a conduit à rechercher si la ribonucléase – enzyme spécifique de ces acides nucléiques – exerce une action sur la division des cellules. Au cours d'une série d'expériences portant sur des œufs d'Amphibiens en voie de segmentation, nous avons montré[2] que la ribonucléase réduite[3] est seule active sur l'embryogénèse; la ribonucléase oxydée[4] au contraire est absolument sans action. Rappelons que, dans ces expériences, des solutions extrêmement diluées de ribonucléase réduite arrêtent la segmentation, tout en modifiant profondément les phosphorylations oxydatives des organismes traités[1]. L'enzyme se révèle donc être *un agent antimitotique* extrêmement puissant.

Des résultats obtenus depuis, à l'aide de préparations de ribonucléase marquée par de l'iode 131[2], démontrent que *les deux formes* (oxydée ou réduite) *de l'enzyme diffusent aisément à travers les membranes cellulaires des œufs de Batraciens.*

Ces résultats permettaient d'espérer que l'on puisse, à l'aide de ribonucléase réduite, atteindre efficacement le métabolisme des cellules cancéreuses et agir sur leur multiplication.

Dans une première série d'expériences, nous avons étudié l'action de la ribonucléase sur des cellules carcinomateuses[3] maintenues *in vitro*. L'action de l'enzyme sur le métabolisme de ces cellules a été suivie. Il ressort clairement de ces expériences que la ribonucléase pénètre dans les cellules et qu'elle agit rapidement sur l'acide ribonucléique qu'elles contiennent.

Les résultats obtenus montrent que le traitement des cellules par la ribonucléase ne modifie pas leur teneur en protéines; mais qu'elle abaisse considérablement leur respiration.

En ce qui concerne l'acide ribonucléique intra- et extra-cellulaire, on observe les phénomènes suivants en présence de ribonucléase réduite par rapport à des témoins traités par de la ribonucléase oxydée:

1° Au cours d'une première phase de l'action, il se produit *une synthèse importante* d'A.R.N. intracellulaire et une augmentation de la teneur en nucléotides libres. Parallèlement, la teneur en nucléotides libres du milieu extracellulaire (liquide d'ascite) diminue rapidement et dans les mêmes proportions.

2° La seconde phase consiste en une *dégradation rapide* de l'acide ribonucléique intracellulaire tandis que la teneur en nucléotides libres reste très élevée. Dans le milieu extracellulaire, on voit réapparaître des nucléotides libres; leur concentration augmente au cours du temps.

On peut conclure de ces faits que la ribonucléase réduite agit en provoquant, dans la cellule vivante, une synthèse importante d'acide ribonucléique et une augmentation de la teneur en nucléotides libres aux dépens du milieu extérieur. Il est vraisemblable que cette synthèse d'A.R.N. et cette accumulation de nucléotides déséquilibrent considérablement l'ensemble des métabolismes cellulaires (cette action semble d'ailleurs être irréversible). La dégradation ultérieure de l'A.R.N. intracellulaire peut s'interpréter comme une action nucléasique banale dans un organisme mort.

Rappelons que l'action de la ribonucléase comme catalyseur de la synthèse de l'A.R.N. vient d'être démontrée par HEPPEL et WHITFELD[4]: ils ont observé une synthèse *in vitro* de polynucléotides à partir de nucléotides cycliques, en présence de ribonucléase.

Les frottis des cellules prélevées à des temps d'incubation différents permettent, eux aussi, de retrouver l'évolution des phénomènes qui viennent d'être décrits: après coloration à l'UNNA, on voit que la basophilie est fortement altérée et qu'après un temps de latence, elle disparaît presque entièrement du cytoplasme. Les microphotos 1 et 2 donnent un exemple des résultats obtenus.

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