Effects of temperature on reptilian and other cells

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INTRODUCTION

Only rarely have reptilian cells been used in cell culture studies. Lewis & Lewis (1925, 1926) investigated the transformation of mononuclear blood cells into macrophages, epithelioid cells and giant multinucleated cells in hanging drop cultures of lower vertebrates, including those of reptiles. Cohen (1926) used hanging-drop cultures of turtle blood to examine the formation of giant cells, because experiments could be carried out at room temperature. He recorded the activity of giant cells for 26 days after initial culture. Chang (1934) examined selachian blood in hanging-drop cultures of whole blood and for comparison made similar studies of other vertebrates, including a snake.

Studies with cell types other than blood cells have been more neglected. Chlopin (1925), studying liver cells of a number of embryonic and adult vertebrates, used a young specimen of Zootoca vivipara, and explanted a liver piece to a hanging drop preparation of rabbit plasma (1–2 months at 22–24 °C). He reported growth and mitosis of fibroblasts and reticulocytes, which he compared histologically with similar elements of other vertebrates. Hibbard (1935) cultivated various tissues of cold-blooded animals in vitro at laboratory temperatures and reported outgrowth of epithelial cells from snake kidney explanted to snake plasma in hanging-drop culture. Grigoriev (1941), using various vertebrates, including some reptiles (viper and lizard), grew gall-bladder epithelium in an unspecified culture medium for 1–1½ months.

The only attempt at reptilian cell culture following tissue disaggregation appears to have been that made by Wolf, Quimby, Pyle & Dexter (1960), who commented on the scant attention to date that had been devoted to the in vitro cultivation of tissues from the lower vertebrates. Wolf applied the techniques he had developed for fish tissues to carry out exploratory work with adult tissues from an amphibian and an aquatic reptile. He reported good results in the attachment on glass and division of cells of the ovary from the painted turtle, Chrysemys picta. Mammalian-type media were used throughout this work, and, depending upon initial density, cell dispersions from reproductive tissue of the fish gave uniform monolayers of epithelial-like and spindle

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cells in 1–6 days at 19 °C. Monolayers of frog and turtle cells formed more slowly.

These previous investigations were not primarily concerned with the cultivation of reptilian cells. The present work was therefore initially designed to test the extent to which disaggregated reptilian cells may be successfully cultured in a partially defined medium known to be suitable for proliferating avian and mammalian cells, and to study the growth and general behaviour of embryonic and adult reptilian cells in culture.

Once reptilian cells were successfully established in culture, attention was given to the determination of the optimal temperature for reptilian cells and the range within which such cells could survive in culture. The investigation was further orientated in an attempt to answer such questions as whether different types of cells from the same animal had different temperature ranges, and whether specific cultured cells behaved differently at different temperatures. Later, the work was made comparative by repeating experiments with larval cells of an amphibian and with embryonic cells of a bird and mammal.

MATERIALS AND METHODS

The skink *Egernia cunninghami* was the main source of adult reptilian cells, though on one occasion cells were obtained from an adult *Amphibolurus diemensis* (Agamidae). Fertile eggs of *A. barbatus* provided the material for most experiments involving cells of reptilian embryos. This was supplemented in later experiments by embryos of *A. gularis*, of the skink *Sphenomorphus ocelliferum*, and of the green turtle *Chelonia mydas*. Cells for comparative studies were provided by 8- or 9-day chicks, approximately 18-day prenatal mice, and tadpoles of the frog *Limnodynastes peroni*.

When cells were required from adult lizards in the preliminary experiments, the animals were killed by decapitation and dissected. The kidneys and gonads were removed and placed in Ca²⁺- and Mg²⁺-free saline. Tissue from these organs was very finely chopped and then trypsinized in a 0·25 % solution of trypsin (Difco Certified 1:250). The suspended material was placed in a centrifuge tube and agitated gently with a pipette. The degree of trypsinization was checked by withdrawing cells for microscopic examination at various stages of disaggregation. When cells were completely dissociated, they were centrifuged at a speed a little less than 1000 r.p.m., washed in Hanks's saline, and then re-centrifuged in readiness for culturing.

Cells were then placed in the culture medium. This consisted of 80 parts Medium 199 (Morgan, Morton & Parker, 1950) with 20 parts foetal calf serum, the whole being buffered by the addition of a small quantity of a 2·8 % solution of sodium bicarbonate. Such a culture medium is known to be adequate to support rapid and continuous growth of avian and mammalian cells for a prolonged period in the laboratory (Morgan, Campbell & Morton, 1955).
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The initial concentration of cells in the culture medium was estimated, counts being made with a haemocytometer. In each experiment this concentration was kept the same.

Cells were cultured directly on cover-glasses. In some cases the cover-glasses were placed each in a Petri dish and a chamber for the culture medium and cells was made by sealing a glass ring on to the cover-glass by means of a hot paraffin/Vaseline mixture. Alternatively, the cover-glass was sealed on to a Cruickshank tissue culture chamber (Cruickshank, Cooper & Conran, 1959) and inverted so that the cells could settle on the cover-glass. With this latter method it was possible to check the concentration of cells after they had been set up in culture by making a count with a squared graticule inserted in the eyepiece of a microscope.

All cultures were set up in vacuum desiccators. They were gassed initially and after every second or third day with an air–CO₂ mixture (5% CO₂). Culture preparations were placed in constant-temperature rooms at 3 °C, 23–25 °C, 31.5 °C or 37–5 °C, or in low-temperature ovens at a selection of temperatures ranging from 37.5 °C to 45 °C. In some experiments, cultures were transferred after a period of cultivation at one temperature to another.

Preliminary experiments were carried out to test whether 80 parts Medium 199 and 20 parts foetal calf serum, a combination essentially designed for the culture of mammalian and avian cells, could be successfully used for the culture of reptilian cells. Cells from the kidneys and gonads of adult skinks (Egernia cunninghami) were set up in culture either at room temperature or at 40 °C.

Several points emerged from these preliminary experiments. First, it was soon found that the adult reptilian cells could be cultured in the mammalian-type medium, used at normal strength. Secondly, the medium proved to be favourable for the cells to spread out on glass. The degree and speed of this behavioural pattern of the cells was furthermore greatly influenced by the temperature. Thirdly, colchicine added to the medium before favourably cultured cells were fixed and stained led to the accumulation of metaphase plates, thus demonstrating that the cells were undergoing mitosis and not merely being maintained in culture. Fourthly, it was found that a constant temperature of 40 °C was very much more favourable than room temperatures for the culture of the reptilian cells. Whole reptilian embryos in culture have recently been shown to be able to survive a considerable range of temperatures (Maderson & Bellairs, 1962; Holder & Bellairs, 1962), but there has been no previous indication that temperatures near the upper extremity of the range would be so favourable for reptilian cells.

Following these preliminary experiments, embryonic cells were used throughout. The embryos for the first series of experiments all came from a single clutch of eggs of Amphibolurus barbatus. The first ones released from the egg were of 6 mm total length; the last ones 15 mm. Entire embryos were finely chopped, trypsinized, and the cells were set up in culture mainly at three temperature
levels: 3 °, 23–25 ° and 37-5 °C. After a period of cultivation at one temperature, some preparations were transferred either from 3 ° to 37-5 °C or vice versa. For comparative purposes, chick and embryonic mouse cells were cultured at 3 °; 23–25 °; 37-5 °; 3 °, later transferred to 37-5 °C; and 37-5 °, later transferred to 3 °C. Also, embryonic reptilian, chick and mouse cells were cultured at a series of high temperatures ranging from 40 ° to 45 °C.

To test its efficacy beyond the range of Amniotes, the medium was used at normal strength for the culture of amphibian cells. Kidney and liver tissue of metamorphosing tadpoles of *Limnodynastes peroni* was either trypsin-dissociated or set up in culture as explant preparations at 31-5 °C or 25 °C.

Towards the final stages of the investigation, fertile eggs of the green turtle, *Chelonia mydas*, became available. This material provided an opportunity of showing the wider applicability of the results with reptilian cells which, until this stage, had been derived solely from diurnal lizards.

The criterion of suitable culture conditions used throughout this study was the general ability of dissociated cells to spread out on glass and to proliferate to form a dense monolayer. Although it was essential to standardize cell concentrations at the beginning of experiments, it was later found to be unnecessary to make quantitative assessments of the cultured cell populations. At the selected temperatures of culture, the differences were obvious from direct observation of the fixed and stained cells.

**RESULTS**

(a) *Suitability of the mammalian-type medium for the culture of reptilian cells*

The results of the experiments clearly show that reptilian cells, whether from adult organs or from entire embryos, may be cultured satisfactorily in a synthetic medium to which is added mammalian serum as an undefined component. Such a mixture is known to be suitable for the prolonged survival and indefinite proliferation of mammalian and avian cells. Reptilian cells are not only maintained in this mixture, which was used at normal strength: under suitable conditions of temperature they will undergo mitosis and proliferate rapidly (Plate 1, fig. C). However, the multiplication of embryonic reptilian cells was not as prolific as that encountered with embryonic mammalian and avian cells, and this situation was not affected by any dilution of the medium. Even at the optimal temperature, a particular degree of monolayer formation could not be achieved in as short a time as with mammalian or avian cells.

The medium was even quite adequate for the maintenance and proliferation of amphibian kidney and liver cells (Plate 1, fig. B). However, the performance of these cultured cells at an optimal temperature of 31-5 °C was inferior to the performance of reptilian cells, cultured sub-optimally at the same temperature, and fell even shorter of that of mammalian and avian cells at 37-5 °C.
(b) Optimal temperature for reptilian cell culture

According to Paul (1959), it is characteristic of warm-blooded animals that their cells are rapidly destroyed at temperatures slightly in excess of those at which they operate best. For mammalian and avian cells (Plate 1, fig. E) the optimal temperature is regarded as being between 37 ° and 38.5 °C.

These general statements may also be applied to reptiles. In the present work, reptilian cells were mainly cultured at 3 °, 23-25 ° and at 37.5 °C. Of these temperatures, 37.5 °C was undoubtedly the most favourable for cell proliferation and for cells to spread out on glass. Furthermore, temperatures even a few degrees higher than 37.5 °C were noticeably less favourable.

No attempt was made in this study to culture cells at a selection of temperatures between 37.5 ° and 31.5 °C, or to establish more precisely the optimal range with cells from different reptiles. However, cells of some diurnal lizards were cultured at 31.5 °C and the results at this temperature were inferior to those at 37.5 °C. On the other hand, exploratory work with chelonian cells indicates that although maximal temperatures are roughly the same as those of diurnal lizards, and although cells may be cultured very satisfactorily at 37.5 °C (Plate 1, fig. D), temperatures a little lower than this may be even more favourable.

The significance of these results is seen partly in relationship to the temperatures selected by other workers. Previously, it seems to have been widely assumed that the optimal temperature for culturing cells of cold-blooded vertebrates would be very much below that for warm-blooded vertebrates. For example, Wolf et al. (1960) did not use temperatures higher than 19 °C in exploratory culturing of dissociated cells of the bullfrog and painted turtle. However, Stephenson & Tomkins (1964) have shown in tissue transplantation studies that reptilian cartilage will grow well at the chick incubation temperature of 37.5 °C, while E. M. Stephenson (1966) has established 30 °C as the optimal temperature in her amphibian organ and cell culture experiments.

c) Viability and behaviour of cells at 3 °C

At 3 °C cells remain viable but do not spread out on glass. This applies to both epithelioid and fibroblast types. Some reptilian cells were kept without change of medium at 3 °C for over a month. Still without change of medium, they were then transferred to 37.5 °C where they proceeded to proliferate and spread out on glass within a day or so of transfer. Avian and mammalian cells also remain contracted, but viable, at 3 °C. In some experiments, after a period of several days to about a week at 3 °C, they were transferred to 37.5 °C, where they followed the same pattern of proliferation and spreading out on glass as seen in reptilian cells.

The effects of delayed cell division caused by some cooling of avian and mammalian cells have already been commented upon by other workers. The same is true for reptilian cells. Apparently cold temperatures provide a block...
to some particular stage of the mitotic process. When temperatures are raised again, there is a rather sudden outburst of cell proliferation.

The spreading out of reptilian, avian and mammalian cells on glass is a remarkable phenomenon in that although the cells can, and obviously do, move across the glass substrate, the spread-out condition itself is irreversible, at least with fairly sudden cooling. If cells which have spread out at 37.5 °C and which have extensive processes are transferred to 3 °C, they cannot round themselves up, and start to deteriorate within a day or so of transfer. They will survive for longer periods if alternately transferred at intervals of a few days from one temperature to the other, but even with this treatment are unable to round themselves off for more prolonged survival at 3 °C.

(d) Behaviour of cells at 23–25 °C

Both epithelioid and fibroblast-type cells spread out at 23–25 °C in reptiles (Plate 1, fig. A), birds and mammals, but compared with more optimal temperatures there is little proliferation. Individual cells or cell groups are usually too sparse to form a continuous monolayer, and this factor alone seems to favour the epithelioid cells. Initially such epithelioid cells may have adopted a fibroblast-like morphology, with processes extended as they move. The general sparseness of cells, however, enables them more readily to come together, assume their more typical morphology and produce conspicuous rounded sheets of epithelial tissue. Paul (1959) points out that some mammalian cells may prefer temperatures lower than 37.5 °C and states that human skin epithelial cells grow better at lower temperatures. This may well be true of reptilian and avian epithelia but a comparison of mitotic rates between fibroblasts and epithelioid cells at 23–25 °C has been beyond the scope of the present investigation. On the other hand a differential response of these two types of cells to high temperatures is more readily shown.

(e) Behaviour and survival of cells at temperatures higher than 37.5 °C

The striking differences seen here are not only between epithelioid cells and fibroblasts in general, but also between avian cells on the one hand and reptilian

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**Plate 1**

Fig. A. Dissociated cells of lacertilian embryo (Sphenomorphus ocelliferum), cultured for 2 days at 25 °C. × 300.

Fig. B. Liver cells of tadpole of Limnodynastes peroni, cultured for 6 days at 31.5 °C. × 180.

Fig. C. Dissociated cells of lacertilian embryo (Amphibolurus barbatus), cultured for 8 days at 3 °C., and then 12 days at 37.5 °C. × 55.

Fig. D. Photograph of an area of turtle embryo cells growing on a cover-glass. The cells were cultured for 3 days at 37.5 °C. × 2.5.

Fig. E. Photograph of an area, slightly smaller than in fig. D., of chick cells growing on a cover-glass. The cells were cultured for 3 days at 37.5 °C., but were of much lower original concentration than the cells illustrated in fig. D. × 2.5.
PLATE 2

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and mammalian cells on the other. The position is made clearer by considering first the effects of high temperatures on chick cells, cultured in all cases for at least 2 days.

At 41 °C chick cells in culture are predominantly of the fibroblast-type morphology but some cells of the epithelioid type occur in rounded clusters. At 43 °C (Plate 2, fig. C) there is still a monolayer of fibroblast-like cells, but it is less dense than at 41 °C and there is a greater tendency for epithelioid cells to group together. At 45 °C (Plate 2, fig. D) fibroblasts are still spread out but are very sparsely distributed between the epithelial clusters after 2 days' culture. At 47 °C no spreading out of cells on glass was observed, and exposure to this temperature for 3 days was found to be lethal to all cells.

The general pattern for reptilian (Plate 2, fig. A) and mammalian (Plate 2, fig. B) cells, again cultured for at least 2 days, is much the same, with epithelial clusters surviving better at higher temperatures than fibroblasts. The performance is such that 40 ° and 41 °C with mammalian and reptilian cells respectively correspond to about 45 °C with avian cells. At high temperatures the results with cells of the diurnal lizards showed no differences from those with cells of the turtle, and in general the performance of reptilian cells was better than that of mammalian. Sparse chelonian fibroblasts were evident at 42 °C, and the upper limit for lacertilian and chelonian cells was about 43 °C. The results are summarized in Table 1.

(f) Comparison of amniote and anamniote cells

Attempts were made to culture amphibian cells under the same conditions as reptilian, avian and mammalian cells, using the normal-strength mixture of Medium 199 and 20% foetal-calf serum. The culturing was carried out at either 31.5 ° or 25 °C. Of these temperatures, the former is nearer the optimal of 30 °C established by E. M. Stephenson (1966), at least for some tissues of the same species of Limnodynastes.

Explants of liver or kidney of the tadpole of L. peroni were placed directly on glass and allowed to settle with very little surrounding medium. The practice adopted by some investigators of setting such explants in a clot of chick plasma and chick-embryo extract was avoided. Such a procedure has the advantage of setting the explant firmly and of facilitating cell outgrowth from it, but it would have provided an undefined nutrient supplement from an avian source.
A primary aim of these experiments was to determine whether or not amphibian cells would grow out on glass, and even proliferate, in the exclusively mammalian-type medium. This, despite the limitations of the technique, they were found to do (Plate 1, fig. B).

It was also found that kidney or liver cells, completely dissociated with trypsin, could be cultured in the medium, which again was found to be adequate for their maintenance and proliferation. Despite this adequacy, the performance of these cells was inferior to that of mammalian, avian and reptilian cells in the same medium. Clearly, the less closely related the material is to the mammalian class, the less suitable is the mammalian-type medium for the culture of its cells. It is strikingly less so for amphibian as compared with reptilian cells.

Table 1. *Culture of embryonic cells at various temperatures*

Figures in parentheses indicate the number of permanent preparations from twenty-seven experimental series upon which the results are based. The minus sign indicates that cells were not expanded; the plus sign that they were normally expanded. The minimum time of culture was 2 days, but where cells were cultured for longer periods of 1 week or more there was no evidence that the increased time had affected the general results.

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<th>Temperature (°C)</th>
<th>Reptilian fibroblasts expanded</th>
<th>Reptilian epithelioid cells expanded</th>
<th>Avian fibroblasts expanded</th>
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<th>Mammalian fibroblasts expanded</th>
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**DISCUSSION**

In the culture of cells of cold-blooded vertebrates, preference seems previously to have been given to the use of balanced salt solutions supplemented with appropriate natural media. Although avian serum and embryo extract have been used successfully for fish and amphibian cell culture by some workers, others have considered homologous media preferable. Instead of chick embryo extract, extracts of fish fry for fish tissues, and extracts of tadpoles for amphibian material, have been used. However, Paul (1959) prophesied that it was not unlikely that synthetic media of the type used for mammalian cells would prove more suitable than these. Wolf et al. (1960), in one of the first studies in comparative cell culture involving cells of lower vertebrates, claimed support for this prediction when they showed that mammalian-type media readily stimulated attachment and division of cells in monolayer cultures from tissues of fishes, and in similar but exploratory cultures from tissues of a frog and a turtle.
This generalization requires qualification in view of more recent results. Shah (1964) observed that so far very few tissues from lower organisms have been grown in the same fashion as those of warm-blooded animals. From a comparison of the growth effects of five different media, he suggested the need of homologous body fluids in media for the culture of amphibian tissues. The mammalian-type medium used in the present study was satisfactory enough in that it permitted cell maintenance and proliferation throughout, but it became progressively less adequate through the vertebrate series from mammalian to amphibian level. It was not as suitable for reptilian as for mammalian and avian cells, and markedly less so when tested with amphibian cells. Thus, in the vertebrate series, there seems to be a gradual shift in the optimal metabolic requirements from class to class, However, the requirements differ not only from class to class, but also for different cells of the one organism.

Stephenson & Tomkins (1964) showed that reptilian, mammalian, and even to some extent amphibian cartilage would grow when xenoplastically grafted to the chick chorioallantois, but that bone from such animals would not, unless from another bird, irrespective of the particular species or even genus. These transplantation experiments were carried out with embryonic materials at stages not subject to known immunological responses. The most probable explanation for these results is that the xenoplastic environment provided for the metabolic requirements of proliferating cells of cartilage but not of bone. A similar explanation, based on the availability of appropriate metabolites, may well be offered for the differential response of certain cells to various culture media. Shah (1964) grew amphibian tissues in different media and found, for example, that fibroblastoid growth of frog heart explants was obtained in media unable to support fibroblastoid growth of newt tissues.

According to Paul (1959), it is characteristic of warm-blooded animals that their cells are rapidly destroyed at temperatures slightly in excess of those at which they operate best. For mammalian and avian cells, the optimal temperature is regarded as being between 37 ° and 38-5 °C. With reptiles, Pasteels (1936) found that chelonian embryonic development was notably accelerated towards 35 °C, and Stephenson & Tomkins (1964) observed that reptilian cartilage will grow on the chick chorioallantois at the incubation temperature of 38 °C. On the other hand, although Maderson & Bellairs (1962) showed that whole reptilian embryos were able to survive, at least for a period, temperatures ranging from 4 ° to 35 °C, they considered temperatures of about 18 °C to be most suitable for the maintenance of the embryos. There has therefore been no clear indication previously that temperatures near the upper extremity of the reptilian range, and essentially optimal for warm-blooded animals, would also be most favourable for the culture of cells of these so-called ‘cold-blooded’ animals.

From the studies of various authors on the survival of lizards at high temperatures, it appears that certain desert species can tolerate temperatures in the vicinity of 40 °C without distress. Warburg (1964) tables the critical thermal
maximal temperature and survival time for six species of Australian lizards. He found that *Amphibolurus reticulatus inermis* (De Vis) can survive 42 °C for 24 h and 44 °C for 8 h. Although there is variation in high-temperature tolerance between species, as also between the different tissues and cells of the same species, there is a close correspondence between the lethal high temperatures of reptilian cells in culture and the heat-death temperatures of the adults. As with mammals, reptilian cells, like the entire animals, are rapidly destroyed at temperatures a little in excess of those at which they best operate.

The maximal temperature of 45 °C, recorded in this study for avian cells surviving in culture for a minimum period of 2 days, is higher than the maximal deep-body temperature recorded for birds by various ornithologists. The highest figure listed in the extensive table of deep-body temperatures of adult birds, compiled by Marshall (1961), is 43·5 °C for *Passer domesticus*.

Amphibian cells grow better at 31·5 ° than at 25 °C. Experiments on amphibian organ culture by E. M. Stephenson (1966) demonstrate that a temperature of 30 °C is optimal, at least for certain cells of *Limnodynastes peroni*, the species used. These results suggest that much of the attempted culture of amphibian cells and organs in the past has been carried out at very much sub-optimal temperatures.

With the embryonic materials used in this study, two primary types of cells were recognized in culture, namely epithelioid and fibroblast-type cells. The epithelioid cells did not necessarily occur in sheets. They could remain through the period of culture as dissociated cells, changing their shape and wandering on the glass substrate with fibroblast-like processes extended. Fibroblast-type cells, on the other hand, never came together to form sheets. The better performance and ultimate survival of epithelioid cells at higher temperatures than the fibroblasts raise new possibilities for the elimination of the latter cells from mixed cultures, or at least for the control of their numbers.

**SUMMARY**

1. Reptilian cells, whether from adult organs or from entire embryos, may be cultured satisfactorily in Medium 199, to which is added 20 % foetal-calf serum.

2. This medium, used at normal strength, is adequate even for the maintenance and proliferation of kidney and liver cells from an amphibian tadpole, but the performance of cells of lower vertebrates in the medium is inferior to that of mammalian and avian cells.

3. When cultured for periods of at least 2 days, avian cells survive in an apparently normal fashion at higher temperatures (maximal, 45 °C) than reptilian; reptilian cells at higher temperatures than mammalian; and mammalian cells at higher temperatures than amphibian.

4. There is a close correspondence between the lethal high temperatures of
embryonic cells of diurnal lizards in culture (42–43 °C) and the known heat-death temperatures of adult animals.

5. Although inactive and contracted in culture at 3 °C, reptilian, avian and mammalian cells remain viable, often for prolonged periods without change of medium.

6. The optimal temperature for cell culture is in the vicinity of 37.5 °C for reptilian, avian and mammalian cells, and about 31.5 °C for the amphibian cells used.

7. Different types of cells from the same animal may have different temperature ranges. Epithelioid cells, for example, function better at higher temperatures than fibroblasts and in some cases may be shown to have a slightly higher maximal temperature.

8. Dissociated epithelioid cells in culture may adopt a fibroblast-type of morphology, wandering on the glass surface with extended processes, or they may come together with other similar cells to form epithelial sheets. Fibroblast-type cells may contribute to monolayer formation, but do not join with epithelioid cells to form epithelial sheets.

RÉSUMÉ

Action de la température sur les cellules de Reptiles et d'autres animaux

1. Des cellules reptiliennes, soit d'organes adultes, soit d'embryons entiers, peuvent se cultiver convenablement dans du milieu 199 auquel on ajoute 20 % de sérum de veau foetal.

2. Ce milieu, à concentration normale, convient même pour le maintien et la prolifération de cellules rénales et hépatiques de tétrard d'Amphibien, mais les résultats obtenus avec les cellules de Vertébrés inférieurs dans ce milieu sont inférieurs à ceux des cellules de Mammifères et d'Oiseaux.

3. Quand on les cultive pendant une période de 2 jours au moins, les cellules d'Oiseaux survivent de manière apparemment normale à des températures plus élevées (maximum 45 °C) que les cellules de Reptiles; celles-ci à des températures plus élevées que les cellules de Mammifères; et celles-ci à des températures plus élevées que les cellules d'Amphibiens.

4. Il y a une correspondance étroite entre les températures élevées des cellules embryonnaires de lézards diurnes en culture (42 à 43 °C) et les températures connues pour entraîner la mort des animaux adultes.

5. Bien qu'inactives et contractées en culture à 3 °C, les cellules de Reptiles, d'Oiseaux et de Mammifères restent viables, souvent pendant des périodes prolongées, sans changement de milieu.

6. La température optimale de culture est au voisinage de 37,5 °C pour les cellules de Reptiles, d'Oiseaux et de Mammifères, et d'environ 31,5 °C pour les cellules d'Amphibiens utilisées.

7. Différents types de cellules du même animal peuvent avoir divers domaines
de température. Les cellules épithélioïdes, par exemple, fonctionnent mieux à températures élevées que les fibroblastes et dans quelques cas peuvent se révéler supporter une température maximale légèrement plus élevée.

8. Des cellules épithélioïdes dissociées en culture peuvent prendre une morphologie de type fibroblastique, émigrant à la surface du verre à l'aide d'extensions, ou bien elles peuvent se grouper avec d'autres cellules semblables pour former des couches épithéliales. Les cellules de type fibroblastique peuvent contribuer à former des couches monocellulaires mais ne s'associent pas aux cellules épithélioïdes pour former des couches épithéliales.

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