TEMPERATURE TOLERANCE OF CULTURED AMPHIBIAN CELLS IN RELATION TO LATITUDINAL DISTRIBUTION OF DONORS

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Summary

Whole-organ and chopped-tissue explants from 4 genera and 7 species of Australian leptodactylids were incubated at temperatures ranging from 4 to 41°C. The donors were obtained from one of three latitudes, approximately 19°S., 34°S., or 43°S. Intraspecific comparison of temperature tolerance was provided by tissues of *Limnodynastes tasmaniensis* from both 19°S. and 34°S.

In general, the biokinetic temperature ranges *in vitro* could be correlated with the locations and latitude ranges of the donors but considerably less variation was found in thermal maxima for tissues of *L. tasmaniensis* from 19°S. and 34°S. than in those for tissues of interspecific donors from the same two latitudes. The thermal maximum for explants from tropical donors (19°S.) lay above 41°C, while for *Crinia tasmaniensis* (43°S.) it lay between 30 and 37°C. Explants from donors from 34°S. had intermediate maxima. Optimal and maximal temperatures were not obviously altered by previous low temperature maintenance of cultures. In all cases, the temperature range supporting functional maintenance and tissue viability exceeded that favouring cell outgrowth. Some evidence of tissue specificity in relation to temperature tolerance was obtained. The results help to explain apparent anomalies in previous records of incubation temperatures used for amphibian cultures.

I. Introduction

It is known (Bělehrádek 1935; Moore 1942*a*; Bullock 1955; Brattstrom 1963) that many poikilotherms, including amphibia, are able to carry out a variety of homeostatic adjustments which can help to make them independent, within limits, of differing environmental temperatures. It is also recognized that poikilotherms of the same or different species occupying different latitudes may also show geographic temperature compensation, e.g. differences in developmental rates or embryonic temperature tolerance (Moore 1942*a*; Volpe 1953). On the other hand, intraspecific exceptions have been demonstrated in certain species of *Rana* (Moore 1942*a*, 1942*b*) and in *Bufo valliceps* (Volpe 1957) in which adaptive changes correlated with differences in latitude have not been found.

Comparatively little information exists regarding either temperature effects on cultured amphibian cells and tissues or on the temperature tolerance of such cultures

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in relation to the geographical locations of the donor animals. The most usual temperatures employed for amphibian cultures by recent workers in the United Kingdom, United States, and Japan appear to range from 25 to 26·5°C (Auclair 1961; Freed 1962; Wolf and Quimby 1964; Seto and Ojima 1966). Drew (1913) showed that explants from the European frog *Rana temporaria* would not survive even 12 hr exposure to 37·5°C. Wolf and Quimby (1964) found that cells of a permanent line from *R. catesbiana* could not tolerate direct transfer from 25 to 37°C. Yet Stephenson and Tomkins (1964) showed that limb-bone rudiments of *Pseudophryne bibroni* from Sydney will grow on chick chorioallantoic membranes at 38°C. It has further been demonstrated (Stephenson 1967) that cultured larval hearts of *Limnodynastes peroni* from Sydney function efficiently and produce optimal outgrowth of cells at 30°C within an incubation range of 5–37°C and can maintain pulsations without cell migration for up to 6 days at 37°C.

These varying results suggested the present investigation in which the biokinetic temperature ranges of amphibian tissues *in vitro* have been considered in relationship to the approximate latitudes from which the actual donors were collected and to the latitude ranges of the donor species. More direct comparison with field conditions cannot be made until adequate information regarding microclimatic and body temperatures is available (Brattstrom 1963).

II. Materials and Methods

Four genera and seven species of Australian leptodactylids with varying latitude ranges (Table 1) were used as donors. Collecting was confined to the east coast of the mainland and to Tasmania. The donors were obtained from one of three localities:

(1) areas around Townsville (approx. lat. 19°S.);

(2) areas around Sydney and extending south to Kiama (34–35°S.);

(3) Mt. Wellington in Tasmania (43°S.). Altitude (3800 ft) was an additional factor presumably affecting temperature in this area.

Intraspecific comparison was provided by the use of *Limnodynastes tasmaniensis* from areas (1) and (2), the Queensland and New South Wales donors being distinguished in this paper as *L. tasmaniensis* (Q) and *L. tasmaniensis* (S) respectively (see Table 1).

Animals from Queensland and Tasmania were sent by air to Sydney and processed within 24 hr of arrival. In most cases the donors were larvae of varying stages but it was necessary to use recently metamorphosed (referred to herein as juvenile) or adult material of *L. tasmaniensis* (S) and *Crinia tasmaniensis*. The possible effects of age differences were considered when assessing results (see later). The variable survival rates of the tropical larvae during transit inevitably influenced the available number of explants and types of tissues cultured. In general, explants included lung, kidney, heart, liver, and occasionally skeletal elements.

Larval explants were prepared as described for hearts of *L. peroni* (Stephenson 1967). Adults were washed in tap water, detergent, and 95% ethanol and wrapped in sterile, saline-soaked gauze before being pithed and dissected. All explants were incubated as lying-drops in cavity slides, using Maximow double coverslips (see Paul 1960). The explants were held in clots of avian plasma and embryo extract. The liquid avian–synthetic medium and the proportions of antibiotics used were similar to those used for the culture of *L. peroni* hearts (Stephenson 1967). Washing in saline and renewal of the liquid medium took place at 2-day intervals except where cultures were maintained at low temperatures for several weeks with minimal disturbance. The average experimental period occupied 1 week but longer or shorter times were used for special purposes.

TABLE 1

GENERAL DISTRIBUTION AND PLACE AND TIME OF COLLECTION OF DONORS*

Species	General Distribution	Approximate Range of Latitude	Collecting Areas	Month of Collection and Culture
Limnodynastes convexiusculus (Macleay)	New Guinea, Groote Eylandt, N.T., Queensland, Northern Territory	7(?)–25°S.	Giru, near Townsville, 19·5°S.	March–April
Cyclorana alboguttatus (Günther)	Queensland, Northern Territory, northern and inland New South Wales	13–28°S.	Townsville, 19°S.	Feb.–March
Limnodynastes ornatus (Gray)	Thurday I., Groote Eylandt, N.T., Queensland, Northern Territory, northern New South Wales	13–33°S.	Townsville, 19°S.	Feb.–March
Limnodynastes tasmaniensis Günther	Queensland, New South Wales, Victoria, South Australia, Tasmania	15·5–43°S.	(Q) Cluden, near Townsville, 19·3°S. (S) Prospect, near Sydney, 34°S.	April–May May
Limnodynastes peroni (Duméril & Bibron)	Queensland, New South Wales, Victoria, Tasmania	17–41°S.	Sydney, 34°S.	May (previously Feb.–Sept.)†
Myxophyes fasciolatus Günther ‡	New South Wales	30–>34·7°S. (?)	Near Kiama, 34·7°S.	May
Crinia tasmaniensis (Günther)	Tasmania, mainly above 3000 ft	41–43·5°S.	Mt. Wellington at 3800 ft, 43°S.	March

* Information based on that given by Parker (1940), Moore (1961), and, for Queensland spp., personal communications from Dr. I. R. Straughan.

† Stephenson (1967).

‡ The name *Myxophyes fasciolatus* is temporarily retained here but work by Straughan (1966) indicates that the species found at Kiama has its northern limit at approx. 30°S. and not in northern Queensland (cf. Moore 1961).

Incubation temperatures ranged from 4 to 41°C. Standard constant-temperature rooms with negligible fluctuation were used throughout except at 41°C, at which temperature a thermostatically controlled incubator was used. Where the numbers (at least five for each temperature of incubation) of suitable explants (e.g. lung) were available throughout the incubation period, approximate quantitative estimates of outgrowth were made using weighed, cut-out projection drawings (see Paul 1960; Stephenson 1967). In all cases the mean bulk area of the explants was converted to a standard weight and the outgrowth weights were adjusted accordingly (Fig. 1).

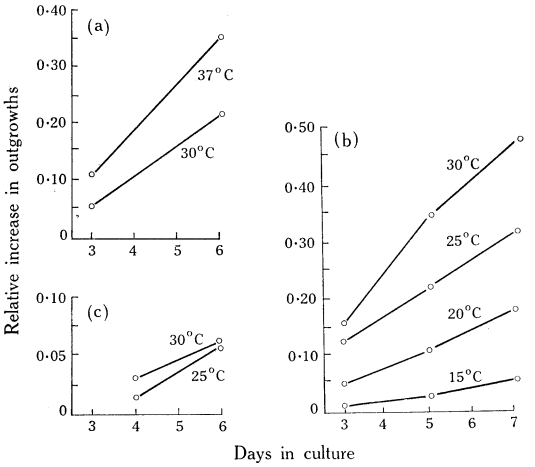


Fig. 1.—Relative increase in outgrowth from lung explants of metamorphosing tadpoles of *Cyclorana alboguttatus* (*a*), tadpoles of *Myxophyes fasciolatus* (see Table 1) (*b*), and *Crinia tasmaniensis* adults (*c*) incubated at the temperatures shown. The comparisons are based on mean weights in grams of projection drawings (×32) on standard paper (10 cm^2 = 0·045 g) of outgrowths from 5 [(*a*) and (*c*)] or 10 (*b*) cultures per temperature. The mean weight of the projection drawings of the initial bulk areas of the explants was standardized at 0·05 g for all temperatures.

Most cultures were fixed in formol saline and stained in Mallory's aqueous haematoxylin. A few were treated with silver nitrate (1 : 400) before fixation. The Jenner–Giemsa technique (see Paul 1960) was occasionally used. Photographs were taken with a Zeiss photomicroscope.

III. Results

(*a*) *Outgrowth*

Detailed results relating to outgrowth from all species are presented in Table 2. Only explants from donors with specifically tropical distributions were able to proliferate at 41°C. At this temperature rapid migration and numerous cell divisions were obtained from explants of *Cyclorana alboguttatus* and *Limnodynastes ornatus* (Figs. 2 and 6), although after 5–7 days patches of degenerating cells (Fig. 3) and a high incidence of vacuolation (Fig. 4) were evident. In contrast, explants from *L. tasmaniensis* (Q) although obtained from a tropical latitude, produced only slight and transient migration at 41°C.

The optimal incubation temperature for explants of most tissues from tropical donors, including *L. tasmaniensis* (Q), appeared to be 37°C [Figs. 1(*a*), 8–10]. Even after 8 days at 37°C, colchicine-treated cultures of *C. alboguttatus* were used successfully for karyotype preparation. At this temperature, however, an apparent indication of tissue specificity in relation to temperature tolerance was provided by liver explants from *Limnodynastes* spp. (Table 2) which produced no outgrowth and gradually became moribund. At 30°C, liver explants from the same species gave rise to healthy cells (Figs. 5 and 11). Of explants from non-tropical donors cultured at 37°C, only those of *L. tasmaniensis* (S) produced outgrowth, the degree of success

TABLE 2

SUMMARIZED CULTURE RECORD FOR ALL SPECIES STUDIED

Incubation Temp. (°C)	No. of Explants	Day of Transfer and Temp.*	No. of Explants which Produced Outgrowth	Types of Explants	Explants which Produced Outgrowth
			Cyclorana alboguttatus		
41	22		20	Kidney, lung, atrium, conus, ventricle (fragmented)	All except two ventricle fragments
37	22		22	As above	All
30	22		22	As above	All
			Limnodynastes ornatus		
41	6	4, 25°C	6	Kidney, heart, T.S. tail	All
41	8	4, 14°C	4	Kidney, lung, vertebrae, T.S. tail	Kidney, lung
37	11		9	Kidney, lung, heart, liver, vertebrae, T.S. tail	All except liver
30	9		9	Kidney, lung, heart, liver, limb buds, T.S. tail	All
25	9		9	Kidney, lung, heart, eyes, T.S. tail	All
14	8		Nil within 4 days	Kidney, lung, vertebrae, T.S. tail	Nil within 4 days
			Limnodynastes convexiusculus		
37	8		6	Kidney, lung, liver, heart	All except liver
37	6	8, 14°C	5	As above	As above
37	7	8, 4°C	3	As above	Kidney, heart
30	8		8	As above	All
25	11		11	As above	All
14	10		Nil within 8 days	As above	Nil within 8 days
4	11		Nil within 8 days	As above	Nil within 8 days

* Temperature to which explant was exposed prior to transfer.

TABLE 2 (*Continued*)

Incubation Temp. (°C)	No. of Explants	Day of Transfer and Temp.*	No. of Explants which Produced Outgrowth	Types of Explants	Explants which Produced Outgrowth
			Limnodynastes tasmaniensis†		
41	20 (Q)		4‡	Heart, spleen, lung, liver, kidney, forelimb	Heart, lung, forelimb
37	20 (Q)		16	As above	All except liver
37	3 (Q)	8, 14°C	1	Heart, liver	Heart
37	7 (Q)	8, 4°C	5	Heart, liver, kidney, forelimb	All except liver
37	38 (S)		27	Lung, kidney, heart	Lung (all), kidney (50%), heart (conus fragments only)
37	14 (S)	28, 4°C	12	Lung, kindey	Lung (all), kidney (50%)
30	15 (Q)		15	Heart, spleen, lung, liver, kidney, forelimb	All
30	5 (Q)	8, 14°C	5	Kidney, liver, forelimb	All
30	4 (Q)	8, 4°C	4	Heart, liver, kidney	All
30	30 (S)		27	Heart, lung, kidney	All except three ventricle fragments
30	2 (S)	28, 4°C	2	Kidney	Kidney
14	10 (Q)		2 by day 8	Heart, liver, kidney, forelimb	Liver, forelimb
4	11 (Q)		Nil by day 8	Heart, liver, kidney, lung,	Nil by day 8
4	17 (S)		1 by day 20	Lung, kidney	Lung
			Limnodynastes peroni		
37	20		Nil	Kidney, lung	Nil
30	18		18	As above	All
30	12§		12	Lung	Lung
30	4	25, 4°C	4	Kidney	Kidney
4	4		Nil within 25 days	Kidney	Nil within 25 days

* Temperature to which explant was exposed prior to transfer.

† Records of cultures from donors from two localities are included, distinguished by (Q) (donors from northern Queensland, 19·3°S.) or (S) (donors from near Sydney, 34°S.) after the number of explants.

‡ Only slight outgrowths produced.

§ Previous series. Recently metamorphosed donors (see Fig. 16).

TABLE 2 (*Continued*)

Incubation Temp. (°C)	No. of Explants	Day of Transfer and Temp.*	No. of Explants which Produced Outgrowth	Types of Explants	Explants which Produced Outgrowth
			Myxophyes fasciolatus†		
37	10		Nil	Lung	Nil
30	20		20	Lung, kidney	Lung, kidney
30	10	4, 37°C	Nil	Lung	Nil
25	20		20	Lung, kidney	Lung, kidney
20	20		20	Lung, kidney	Lung, kidney
15	20		20	Lung, kidney	Lung, kidney
			Crinia tasmaniensis		
37	18		Nil	Juvenile kidney, heart, liver, lung. Adult lung, heart, kidney	Nil
37	9	13, 14°C	Nil	Juvenile kidney, heart, lung. Adult lung, heart, testis	Nil
30	25		25	Juvenile kidney, heart, liver, lung. Adult lung, heart, kidney, testis	All
30	5	1 (20 hr), 37°C	Nil	Juvenile kidney, heart. Adult lung, kidney	Nil
30	6	12, 4°C	6	Juvenile kidney, heart. Adult lung, heart, testis	All
25	25		25	Juvenile kidney, heart, liver, lung. Adult lung, heart, kidney, testis	All
25	5	1 (20 hr), 37°C	Nil	Juvenile kidney, heart, lung	Nil
14	21		10	Juvenile kidney, heart, liver, lung. Adult lung, heart, kidney, testis	All except juvenile heart
4	21		1	Juvenile kidney, heart, liver. Adult lung, heart, kidney, testis	Adult lung

* Temperature to which explant was exposed prior to transfer.

† See third footnote, Table 1.

apparently depending to some extent on the tissues used (Table 2). Tissues of *Crinia tasmaniensis* could not tolerate exposure to 37°C for even 20 hr without permanent damage to explants and to previously migrated cells (Fig. 22).

At 30°C all types of tissues from all three latitudes produced adequate outgrowth (Figs. 5, 11, 13, 16, 18, and 23). Both quantitatively and qualitatively 30°C was probably suboptimal for most tissues from tropical donors [Fig. 1(*a*)] and, at least on a long-term basis, for those from *Crinia tasmaniensis* [Fig. 1(*c*)]. In both adult and juvenile tissues of *C. tasmaniensis* some evidence of cell degeneration was apparent after 1 week at 30°C and the rate of lung outgrowth tended to drop relative to that of explants cultured at the apparent optimum of 25°C [Figs. 1(*c*) and 24]. Optimal results for tissues of *L. peroni* (Fig. 16) and *M. fasciolatus* [Figs. 1(*b*), 19–21] were obtained at 30°C.

Although cultures from the tropical donor *L. convexiusculus* grew only slowly at 25°C, adequate migration was ultimately obtained (Fig. 7). Explants from tissues of the specifically tropical species *L. ornatus* and *L. convexiusculus* produced no cells at 14°C within the restricted incubation period of 4–8 days (Table 2). Within 8 days, however, limited fibroblast migration was obtained from tissues of *L. tasmaniensis* (Q) at 14°C. Explants from New South Wales and Tasmanian donors gave rise to clearly defined though relatively slow outgrowth at 14 or 15°C (Figs. 21 and 25).

At 4°C blood cell migration from juvenile kidney explants of *Crinia tasmaniensis* occurred within 1 week (Fig. 26). No tissue from any member of the eight groups of donors gave rise to any other type of cell migration within the normal incubation period of 7–8 days. After 20 days, definite outgrowth of epithelioid cells had begun in one adult lung explant of *L. tasmaniensis* (S) (Fig. 15) and after 12 days in one adult lung explant of *Crinia tasmaniensis* (Fig. 27).

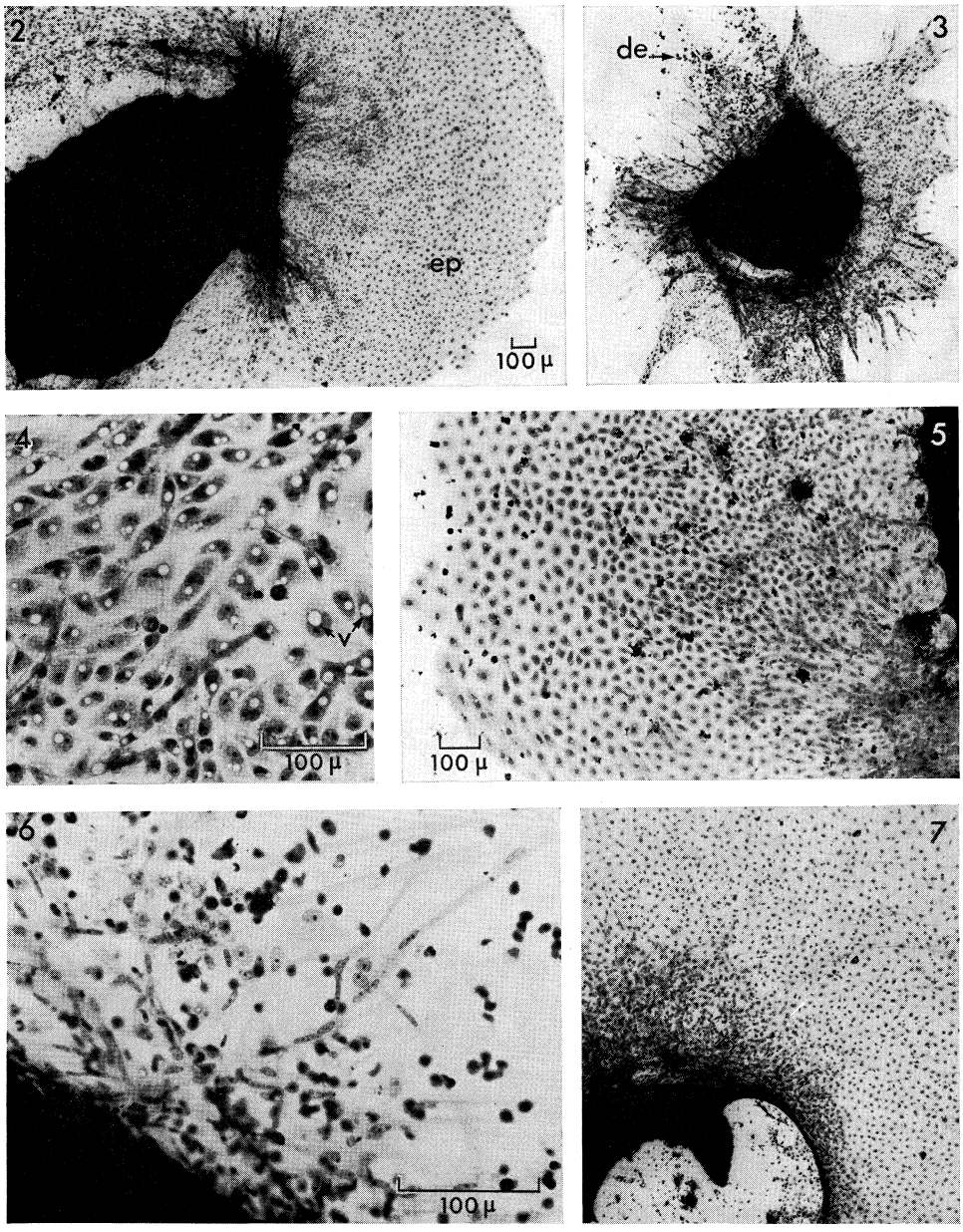
(*b*) *Maintenance of Viability at Temperatures Unsuitable for Outgrowth*

Survival was assessed by (i) direct observation of function, e.g. heart pulsations or ciliary beating, and (ii) by noting the capability of any type of explant to produce cell outgrowth when transferred from an unfavourable to a known favourable temperature.

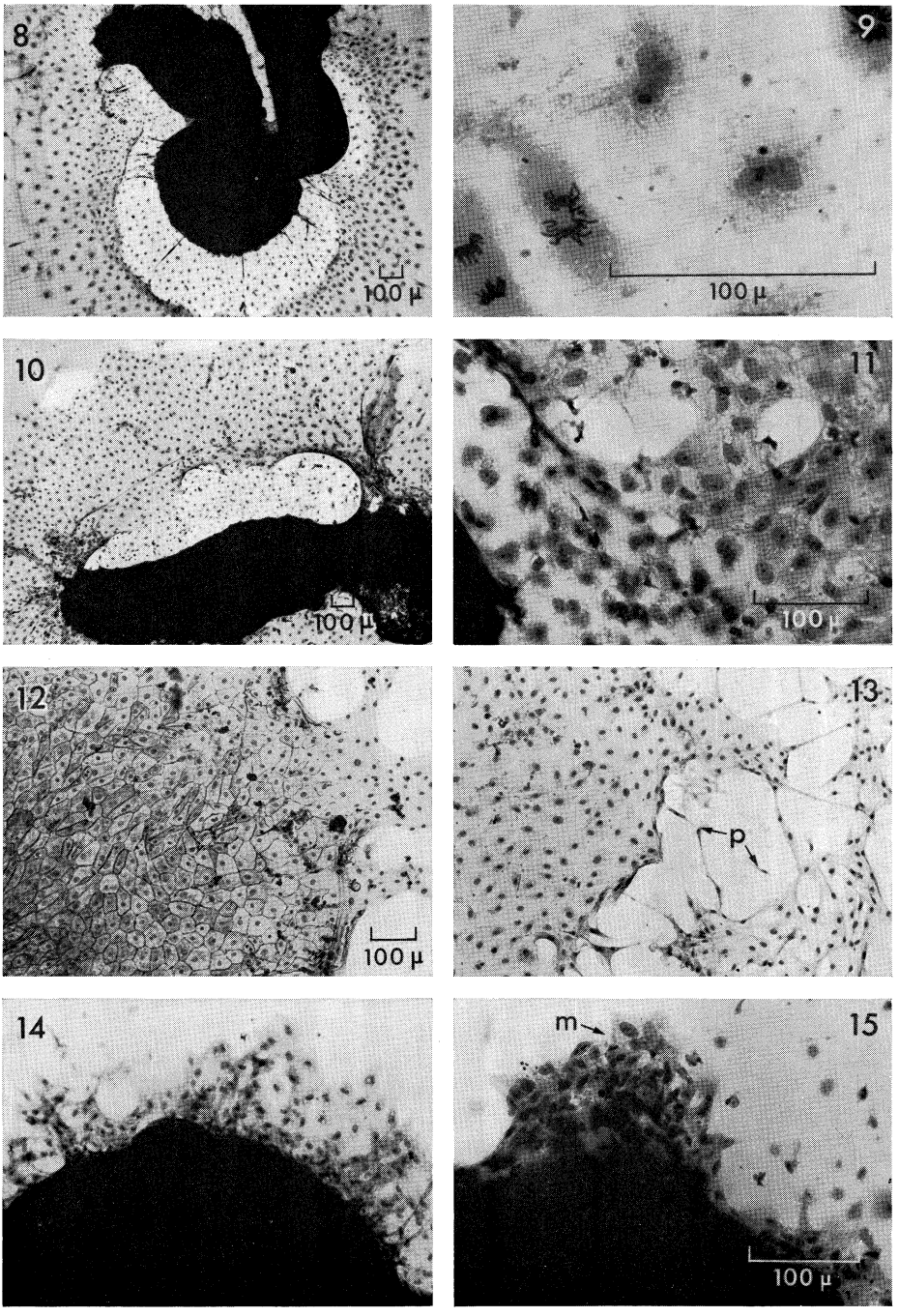
(i) *Direct Observation of Function*

(1) *Heart Pulsations.*—The influence of temperature on heart beat rates *in vitro* was previously established for *L. peroni* (Stephenson 1967). As the highest incubation temperature used in the present experiments permitted cell outgrowth in addition to pulsations of hearts from tropical donors, it was not possible to assess an upper thermal limit for temporary maintenance of function as distinct from cell migration (Stephenson 1967). In *L. tasmaniensis* (Q), however, in which outgrowth was very limited and short-lived at 41°C, hearts continued to beat for only 3 days. Juvenile hearts and adult heart fragments of *Crinia tasmaniensis* all stopped beating within 20 hr at 37°C and did not resume after transfer to 30 or 25°C, indicating that the thermal maximum for heart function of this species *in vitro* lies between 30 and 37°C.

Hearts of all species cultured at 14°C, regardless of geographical origin, maintained slow but apparently continuous beating for at least 7–8 days. Hearts of



Figs. 2–7.—Cultures of tissues from three tropical donors: 2, *Cyclorana alboguttatus*, lung, 2 days at 41°C, showing healthy epithelioid outgrowth (*ep*); 3, *C. alboguttatus*, lung, 5 days at 41°C, showing area of degeneration (*de*); 4, *C. alboguttatus*, lung, 5 days at 41°C, showing vacuolated cells (*v*); 5, *L. ornatus*, liver, 4 days at 30°C; 6, *L. ornatus*, mixed outgrowth from kidney after 4 days at 14°C without outgrowth, followed by 4 days at 41°C; 7, *L. convexiusculus*, lung, 8 days at 25°C. Magnifications for Figures 3 and 7 as for Figure 2.



Figs. 8–15.—Cultures of tissues of *L. tasmaniensis* from donors at latitudes 19°S. (8–11) and 34°S. (12–15). 8, *L. tasmaniensis* (Q), whole heart of larva, after 8 days at 4°C without outgrowth, followed by 2 days at 37°C. 9, *L. tasmaniensis* (Q), cells from the same culture as in 8, showing

L. convexiusculus stopped beating within 24 hr at 4°C but resumed whenever exposed for 10–15 min to a room temperature of 25°C. After transfer to 37°C on day 8, continuous beating was again initiated. Hearts of *Crinia tasmaniensis* maintained regular pulsations for variable periods up to 12 days at 4°C. All hearts and heart fragments of this species resumed beating after transfer to 30°C on day 12.

(2) *Ciliary Beating.*—Cilia were particularly large and obvious in the open nephrostomes of many kidney explants. They were also noticeable in epithelial cells migrating from some lung explants. As with heart pulsations, the upper thermal limit for maintenance of ciliary beating in explants from tropical species lay above the highest incubation temperature employed. At 4°C ciliary beating was maintained indefinitely in appropriate explants of donors from all latitudes. It continued after direct transfer to markedly higher temperatures, e.g. beating which was continued for 8 days at 4°C in kidney explants of *L. convexiusculus* and *L. tasmaniensis* (Q) continued unchecked after transfer to 37°C. Cilia of adult lung of *L. tasmaniensis* (S) beat for 28 days at 4°C and continued at 37°C. Nephrostome cilia of *Crinia tasmaniensis*, which beat continuously for 8 days at 4°C, survived transfer to 30°C but were permanently inhibited in less than 20 hr at 37°C.

(ii) *Outgrowth Following Transfer from an Unfavourable to a Favourable Temperature*

Table 2 provides examples of transfers from those temperatures at which outgrowth did not occur to temperatures known to support migration. In almost all cases, maintenance at low temperatures for variable periods did not affect the ability of cells to migrate after transfer (Figs. 6 and 14). In cases where specific reactions of different tissues to temperature had been noted in routine cultures [see Section III(*b*)(ii)], similar results were obtained for *L. convexiusculus* and *L. tasmaniensis* after transfers from low temperatures (Table 2). Transfers of explants of *M. fasciolatus* and *Crinia tasmaniensis* (Table 2) from 37°C to typically favourable temperatures failed to produce any cells (cf. Stephenson 1967).

(*c*) *Morphological Comparison of Cultures*

Lung and kidney cultures of *M. fasciolatus* (Table 2), of which relatively larger numbers were investigated compared with other genera of the present series, provided distinct contrasts in total outgrowth morphology and in consequent suitability for quantitative comparisons.

In lung cultures, at temperatures from 15 to 30°C, a clot-free space developed adjacent to the explant during early cell migration. Over this, elongated cells or cell groups stretched from the upper levels of the explant into the edge of the clot

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divisions at 37°C. 10, *L. tasmaniensis* (Q), lung, 4 days at 37°C. 11, *L. tasmaniensis* (Q), liver outgrowth, 4 days at 30°C. 12, *L. tasmaniensis* (S), lung outgrowth, treated with silver nitrate before fixation, 3 days at 37°C. 13, *L. tasmaniensis* (S), lung outgrowth, treated with silver nitrate, 5 days at 30°C, showing peripheral cells (*p*) breaking away and changing form. 14, *L. tasmaniensis* (S), lung explant, 28 days at 4°C without migration, followed by 2 days at 37°C, showing cell outgrowth. 15, *L. tasmaniensis* (S), lung, migration (*m*) beginning after 20 days at 4°C. Magnifications for Figures 13 and 14 as for Figure 12.

(Figs. 20 and 21). At 25–30°C, the threads were rapidly broken and the culture soon consisted of an unbroken epithelioid layer covering the clot-free area and extending well beyond it onto the clot itself (Fig. 19). This type of monolayer was clearly defined and proved suitable for outline projection drawing followed by measurement [Fig. 1(*b*)].

Successive drawings were also made of cultured kidneys of *M. fasciolatus* but were insufficiently accurate for quantitative comparisons. This was because of the continuous emergence of thickly packed blood cells which formed a dense mass around the explants before definitive outgrowth was established (Fig. 17), and later partly obscured the latter. Many of the blood cells became loose in the medium and were variably reduced in numbers during each washing of the cultures. They were typically rounded but irregular forms also occurred (cf. Fig. 26). The rounded forms presumably represented lymphoid haemoblasts (Jordan 1933) or stem cells (Foxon 1964) from which the morphologically identifiable types of blood cells develop.

Typical kidney explants of all species were surrounded by a mixture of fibroblasts and epithelioid cells, intermingled with blood cells (Figs. 6 and 18). Outgrowth occurred at a number of levels, making the edges difficult to define.

IV. Discussion

The results indicate that the geographical locations of donor animals should be considered when establishing incubation temperatures for amphibian tissues. They also provide a probable explanation for the apparently conflicting observations regarding temperature effects on amphibian cultures made by Drew (1913), Stephenson and Tomkins (1964), Wolf and Quimby (1964), and Stephenson (1967). Although the present experiments have shown that explants from tropical species were able to survive and produce outgrowth at suboptimal temperatures, most tissues were obviously better suited by an incubation temperature of at least 37°C and could produce extensive though less enduring outgrowth at 41°C. In contrast, 37°C was a rapidly lethal temperature for *Crinia tasmaniensis* from a latitude of 43°S.

When diagrammatic comparison is made of the results from all species and latitudes (Fig. 28) it is seen that the behaviour of tissues *in vitro* bears a direct relationship, at least as far as optimal and maximal incubation temperatures are concerned, to the latitude from which the donors were collected. It is also clear that in every case, as previously established for *L. peroni* hearts (Stephenson 1967), the temperatures permitting maintenance of tissue viability were greater in range than those which also favoured cell migration.

Results from cultures of *L. tasmaniensis* (Q) from 19°S. and *L. tasmaniensis* (S) from 34°S. had a closer mutual resemblance than did those from interspecific donors from the same two areas (Fig. 28). In addition, each of the two sets of results [(Q) and

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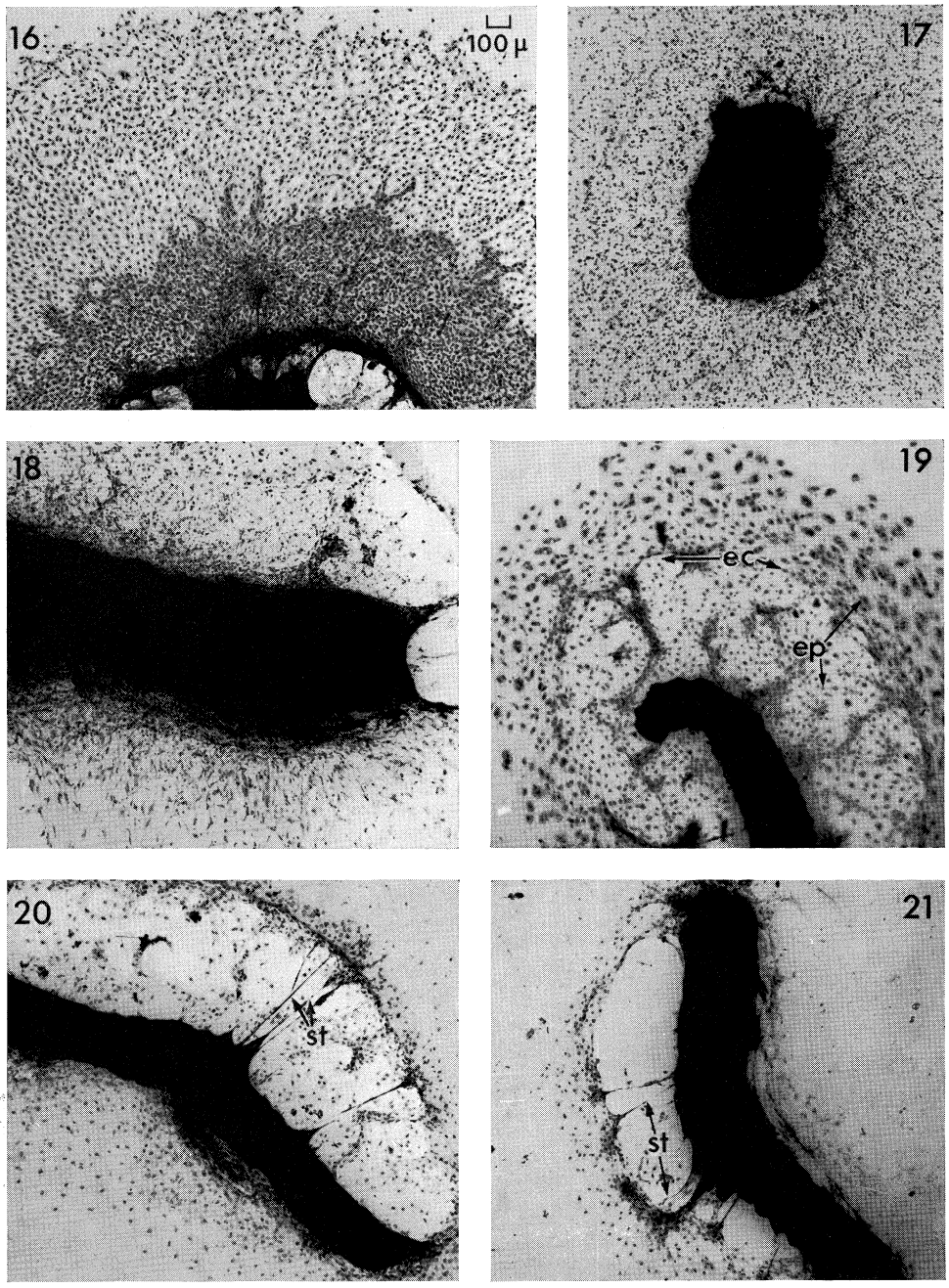
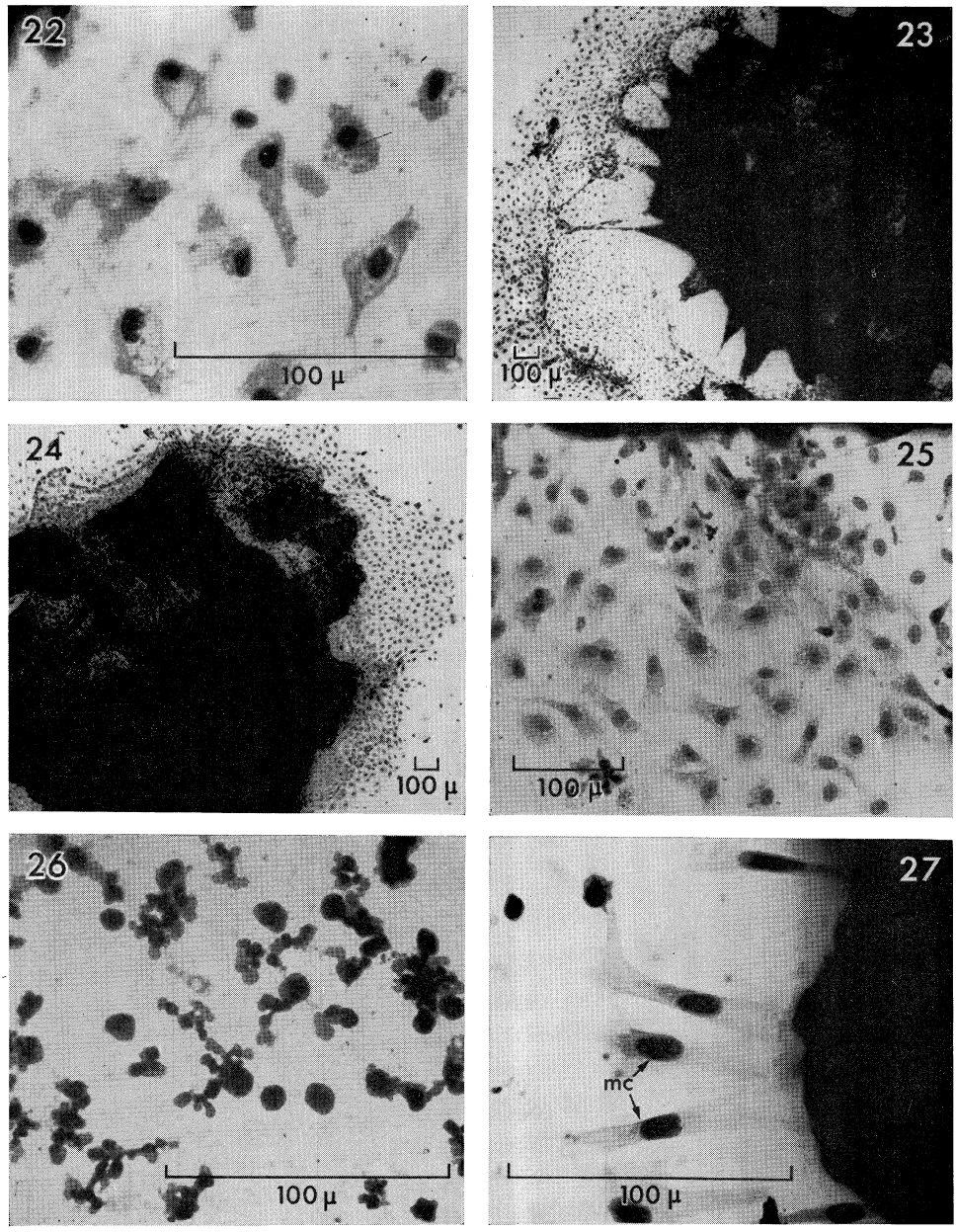
outgrowth, 8 days at 30°C. 19, *M. fasciolatus*, half of larval lung showing epithelioid monolayer (*ep*), 8 days at 25°C. *ec*, edge of clot. 20, *M. fasciolatus*, larval lung, with a few bridging strands of cells (*st*) still intact, 8 days at 20°C. 21, *M. fasciolatus*, larval lung with bridging strands (*st*) and very slight outgrowth, 8 days at 15°C.



Figs. 16–21.—Cultures of lungs and kidneys of *L. peroni* and *M. fasciolatus* from 34 to 35°S. All magnifications as for Figure 16. 16, *L. peroni*, part of outgrowth from lung fragment of recently metamorphosed frog, 4 days at 30°C. 17, *L. peroni*, fragment of larval kidney showing migration of blood cells, 2 days at 30°C. 18, *M. fasciolatus*, part of larval kidney showing mixed



Figs. 22–27.—Cultures of tissues of *Crinia tasmaniensis* from 43°S.: 22, degenerating cells of outgrowth from juvenile kidney, incubated for 13 days at 14°C, followed by 2 days at 37°C; 23, part of adult lung explant and outgrowth, 7 days at 30°C; 24, part of adult lung explant and outgrowth, 7 days at 25°C; 25, part of cell outgrowth from adult lung, 12 days at 14°C; 26, migrating blood cells from juvenile kidney, 10 days at 4°C; 27, migrating cells (*mc*) from adult lung after 12 days at 4°C.

(S)] from this species differed in certain obvious respects from those of other donor species from the same latitude. Tissues of *L. tasmaniensis* (Q), although having an optimal incubation temperature of 37°C, differed from those of other tropical donors in producing only very sparse and short-lived cell outgrowth at 41°C. Lung tissue and, to a lesser extent, kidney and heart of *L. tasmaniensis* (S) (Table 2) gave rise to extensive migration at 37°C, although outgrowth at this temperature was totally absent from explants of *L. peroni* and *M. fasciolatus* from the same general latitudinal zone of 34–35°S. Prior maintenance of explants of *L. tasmaniensis* (S) for 28 days at 4°C produced no change in results following transfer to 37°C.

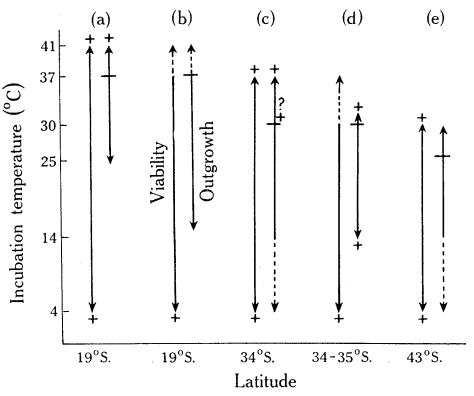


Fig. 28.—Diagrammatic comparison of biokinetic temperature ranges *in vitro* of tissues from donors from three latitudes. (*a*), tropical forms, except *L. tasmaniensis* (Q) (19°S.); (*b*), *L. tasmaniensis* (Q) (19°S.); (*c*), *L. tasmaniensis* (S) (34°S.); (*d*), *L. peroni* and *M. fasciolatus* (34–35°S.); (*e*), *Crinia tasmaniensis* (43°S.). The temperature range in which viability is maintained is shown on the left, and the range permitting outgrowth on the right. – – – – Sparse or infrequent outgrowth or temporary viability. + indicates that limit has not been determined. Optimal temperatures for outgrowth are shown by cross-bars.

As has already been shown (Moore 1942*a*, 1942*b*; Volpe 1957), members of the same amphibian species collected from different latitudes may fail to show differences in embryonic temperature adaptations. When such differences are present intraspecifically they are typically associated with other features suggestive of speciation processes (cf. Moore 1942*a*). In the case of *L. tasmaniensis* [(Q) and (S)], considerable similarity of tissue behaviour *in vitro* was nevertheless linked with apparent inability of specific tissues of *L. tasmaniensis* (S) to achieve total survival at 37°C (Table 2), and with the sparse and possibly selective outgrowth of tissues of *L. tasmaniensis* (Q) at 14°C. A complication which must be considered is the age difference between donors in the two localities, those of *L. tasmaniensis* (Q) being larvae and those of *L. tasmaniensis* (S) young adults. Although this factor cannot be discounted without further investigation, work with other donors of different ages (e.g. *Crinia tasmaniensis*, Table 2) has not demonstrated behavioural differences *in vitro*. Nor has it apparently been shown that increased tolerance of high temperatures is necessarily an accompaniment of increasing age in poikilotherms (Bělehrádek 1935). A further investigation of *L. tasmaniensis*, embracing its full geographical range (Table 1) and including a study of donors in the field, together with their cultured tissues, would almost certainly be rewarding.

The question also arises as to why explants from Sydney donors of *L. peroni*, of which the species distribution pattern is only slightly less in latitude range than *L. tasmaniensis*, are capable of only temporary survival with no outgrowth at 37°C. A possible answer has been supplied by observations of Dr. I. R. Straughan (personal communication) regarding differential breeding localities and water temperatures of

the two species in northern Queensland. Again, more extensive comparisons, *in vivo* and *in vitro*, of *L. peroni* from successive areas would be desirable.

A temperature of 41°C is probably the highest yet recorded for successful culture of amphibian tissues but the actual thermal maximum for tissues of the tropical species involved (Fig. 28) is obviously greater than the highest incubation temperature provided in the present experiments. Dr. I. R. Straughan (personal communication) has recorded survival of tadpoles of *L. ornatus* in water up to 44°C n the laboratory.

The thermal maximum *in vitro* for tissues of *Crinia tasmaniensis* lies between 30 and 37°C (Table 2), with a probable optimum of approximately 25°C. Tasmanian Weather Bureau records obtained by Dr. J. Hickman indicate that from 1961 until March 1967, the maximum air temperature recorded 3 ft above the ground on the summit of Mt. Wellington (3800 ft, 43° S.) only twice exceeded 26·1°C and rarely exceeded 21°C. At the time of collection of specimens, water temperature was 12°C. In captivity, juvenile and adult frogs remained extremely active for several weeks in a constant-temperature room at 14°C. In view of these data it is perhaps surprising that more rapid and extensive outgrowth was not produced at low temperatures by tissues of *C. tasmaniensis*, but even under optimal conditions rate of outgrowth (possibly influenced by age) was markedly lower than that of similar explants from other species (Fig. 1).

In general, regardless of the geographical origin of the donors, maintenance at a low temperature for varying periods did not affect the ability of cells to migrate when transferred to known favourable temperatures. In cases where heart beats ceased at 4°C, they were quickly resumed after transfer to warmer conditions. Ciliary beating was apparently continuous at 4°C.

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