PHYSIOLOGICAL STUDIES ON HARD TICKS

IXODIDAE

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INTRODUCTION

Many authors have stressed the importance of adaptive phenomena observed in the behaviour of host seeking ticks and in their development off the host after engorgement. Alfeev (1948) noticed that adults of Dermacentor pictus Herm., 1804 (=reticulatus) were negatively geotactic in spring and very active, while in midsummer they reversed this behaviour and were not found at the tips of the vegetation. This species and D. marginatus Sulz., 1776, both differed in their behaviour and development in different localities. In a cooler region within their distribution area there was no apparent reversal of behaviour, but a gradual depletion in the stock of ticks by June and July. Adults feeding in summer did not lay eggs until the following spring, and where an appreciable number of adults were active in autumn oviposition was again delayed. There was a true diapause in the engorged female, which persisted even at laboratory temperatures favourable for development, and only after a period of chilling was the preoviposition period comparable with that of spring fed individuals. In another area he noticed that ticks which attached to cattle in midsummer did not always feed, but remained unaltered in size for several weeks. Inability to feed, inactivity and delay in oviposition were all considered as manifestations of seasonal diapause which prevented the appearance of hungry larvae at unfavourable times of the year. Laboratory observations showed that larvae of both species had a poor capacity for survival (Alfeev, 1948, 1954).

/Yashkul

Yashkul (1961) is not entirely in agreement with this opinion but believes that midsummer inactivity of adult D. marginatus is less important than delayed oviposition in synchronising development of the tick. Their avoidance of high temperatures and low humidity in controlled experiments in the laboratory suggested that survival during adverse conditions was the advantage derived from their reversed behaviour (Yashkul 1960). Serdjukova (1960) has emphasised the importance of diapause for winter survival in several Ixodid species although she was interested primarily in the more northerly species Ixodes persulcatus, Schulze, 1930.

Delay in oviposition in D. marginatus is known to be controlled by the daylength experienced by the unfed adults (Belozerov 1963, 1965, Belozerov and Kvitko 1965). Daylength is a reliable indicator of seasonal change and among insects many seasonal adaptations are under its control (Lees 1955, Danilevskii 1965). This is the first Ixodid species in which daylength is known to play such a role. Belozerov (1964a) argues that D. marginatus is a typical short day species. Long photoperiods and high temperatures experienced by the unfed adult induce diapause, while short photoperiods and low temperatures prevent it. Diapause was broken by chilling the engorged or unfed adults at 5°C irrespective of the daylength conditions (Belozerov 1964a, 1964b). These results are readily reconciliable with the field data. Diapause was prolonged in summer, shorter in autumn and absent after experience of low winter temperatures (Alfeev 1948). Comparable seasonal changes in the duration of diapause have been found in D. pictus (Razumova 1965). Belozerov also agrees with Alfeev that the importance of midsummer

/inactivity

inactivity is in synchronising the life cycle with the seasons. He believes that photoperiod controls this form of diapause. The daylength (15 hours) is the same when the ticks disappear in early summer and when they reappear in late summer, but the temperature is much higher at the later date. All the factors involved in midsummer inactivity are not yet fully understood, nevertheless a clear picture of the life history of this tick can be drawn from the observations of these authors.

Among Nearctic species the life history appears to be more complex. In D. andersoni Stiles 1908, adults are inactive in summer (Philip 1937, Mail 1940) : not only do they fail to quest but if placed on a host they are reluctant to attach and fail to engorge (Hunter and Bishopp 1911, Hooker, Bishopp and Wood, 1912). In autumn when conditions of temperature, humidity and photoperiod are again comparable with spring, no recrudescence of activity is found and attachment and feeding are suppressed from August to December (Gregson, 1936, 1938). Gregson (1951) has suggested that decreasing photoperiods are less favourable for these processes than increasing ones but he notes that in drier regions activity ceases in mid-May. Cooley (1911) reported activity of adults at higher altitudes in August and September. At lower stations Cooley (1932) found no activity in autumn of newly emerging adults and nymphs. He considered midsummer inactivity as an avoiding reaction to unfavourable conditions and autumn inactivity as an advantageous adaption preventing exposure of the younger

/stages

stages to winter conditions.

In D. variabilis (Say 1821) activity continues throughout the summer, but in autumn when temperatures and humidity are comparable with spring no ticks are found (Smith, Cole and Gouck, 1946). Earlier observations (Smith and Cole, 1941) suggested that attachment and feeding of larvae and nymphs under laboratory conditions could be improved during the autumn and winter months if they experienced artificially increasing photoperiods. Fixed long photoperiods were also effective but the direction of change was most important. Adults at these same conditions failed to respond to the increasing or long photoperiods and were still reluctant to feed. Activity itself was not observed under artificial conditions. In their later observations (Smith et al. 1946) low temperatures were harmful to the engorged ticks but only the engorged females and the eggs were particularly sensitive. Autumn inactivity prevented exposure of engorged ticks and eggs to winter conditions.

Much remains obscure concerning the behaviour of the New World species, although recently there has been revived interest in several laboratories. There is an interesting parallel between D. andersoni and the Old World species D. marginatus. The seasonal course of activity depends to some extent upon the altitude (Melnikova, 1961). Decline in activity occurs two months earlier (May) at 400-700 metres than at 1200-1500 metres (July) and recrudescence of activity about two months later at the lower altitude. There is evidence

/that

that temperature as well as photoperiod may be involved in the cessation of activity. Clearly each species in its different localities has its own adaptive requirements.

In the genus Ixodes, adaption of the life cycle to the seasons has not been associated with the behaviour of the adults as has been inferred for D. marginatus. The adaption in these ticks is usually a developmental diapause. An autumn diapause is known for the eggs, larvae and nymphs of I. ricinus (Linnaeus 1758) and I. persulcatus, (Kheisin, Pavlovskaya, Malakhova and Rybak 1954; Kheisin, Botchkareva, Lavrenenko and Mikhailova 1954; Babenko 1956) and for the larvae of I. apronophorus Schulze, 1924, (Rubina and Babenko, 1963). It can be inferred from the data of Smith (1945) for the larvae and nymphs of I. dentatus Marx, 1899. The variable and protracted nature of development in the laboratory noted for other species suggests that the phenomenon is widespread in this genus. Diapause phenomena in the genus Haemaphysalis appear to be intermediate in character between those of the other two genera. Delay in oviposition as in Dermacentor was found in Haemaphysalis punctata Can. and Fanz., 1877, by Pomerantzev (1950) and in H. flava Neumann, 1897 by Saito (1960). Delay in development of larvae and nymphs as in the genus Ixodes was found in these two species by the same authors.

Diapause has been extensively studied in I. ricinus. A delay in morphogenesis associated with the autumn season was observed by Kossel et al. (1903), by Bienarovic (1907), by Macleod (1932) and others. Diapause in larvae and nymphs was

/recognised

recognised as a prolongation of the mobile post-feeding stage by Falke (1931) but its significance in regulating the seasonal cycle remained obscure for some years.

There has been in the past considerable speculation on the duration of development under field conditions. Wheeler (1899) observed in Northern England two seasonal peaks of host seeking activity, one in spring and again in autumn. He supposed that the ticks which fed in spring completed their development during the summer and the new instars were the sole constituents of the activity peak in autumn (Two brood theory). This theory was extended by Milne (1945). He believed that in the long period of moderate temperatures in the micro-habitat during autumn, winter and early spring, engorged ticks could develop to the next instar and be ready to feed by spring.

The problem of natural development was not clarified until ticks had been confined under field conditions. Campbell (1946) working in Scotland and Alfeev (1947) in the Leningrad district of the U.S.S.R. found that ticks which fed in spring, developed during the summer, but the next instars, although they emerged in the same autumn, were not usually ready to feed until the following spring. Autumn fed larvae and nymphs together with autumn laid eggs overwintered in diapause. During the cold of winter diapause was broken and development resumed in the higher spring temperatures. In the Scottish population the next instars were ready to feed by autumn (Campbell 1948). Diapause in all stages could not be averted in the laboratory at temperatures normally associated with development, but was broken by chilling.

/Comparable

Comparable life histories have been found in other parts of Europe. Although particular areas have their peculiarities, the main differences are a prolongation of the life cycle and a midsummer peak of activity in the Northern latitudes due to the short summer season. Thus in Austria the life cycle is normally completed in 1.5-2 years and possibly even in one year under the most favourable conditions (Loew, Radda, Pretzmann and Groll, 1963). Farther north in the Lithuanian S.S.R. the life cycle required three years for completion, and the nymphs and adults had normal spring and summer peaks of activity. The larvae began activity only in the third decade of May, reached a peak in June, declined in July and were virtually absent thereafter (Kadite 1961). Within the British Isles the normal pattern in upland pastures is a three year life cycle with activity peaks for all stages in spring and autumn with few or no active ticks in midsummer (Campbell 1948). There are however some local deviations; no autumn activity is found in parts of South East Scotland (Campbell 1948) or in parts of North East England (Milne 1945). Only a midsummer peak was found on several high farms (Hill o' Fare) in North East Scotland (Hendrick, Moore and Morrison, 1938). In East Anglia Barnett (1965) found a midsummer peak of larvae, though somewhat later than the similar phenomenon noticed by Kadite. None of these peculiarities has so far been adequately explained.

In the Moscow region of the U.S.S.R. there was a three year life cycle but again there was a distinction between the larvae and the other instars (Babenko 1956). Although all

/stages

stages had two peaks of activity, the second larval peak declined earlier than the rest and from August onwards none were found. Adults which emerged from spring fed ticks were soon willing to feed at this time of year, but 80-90% of the larvae were quite unwilling to do so. Both Babenko (1956) and Kadite (1961) found that the few larvae which fed in autumn usually failed to survive the winter; all other stages, fed or unfed, were unharmed by winter conditions.

In the Leningrad district the life cycle is not less than three years and between three to six years have been recorded (Alfeev 1947). Farther north in Karelia there is a single peak of activity in midsummer (Kheisin et al. 1954b). Kheisin (1954) also found in some larval batches brought into the laboratory in autumn that few ticks were willing to become active or attach to the host. After two to three months some larvae were willing to attach, others still refused. He did not observe death of engorged larvae, on the contrary he considered diapause in them as an adaption for winter survival.

Campbell (1948) found larvae which emerged in autumn quite unwilling to feed, but by November or December nearly all fed successfully on hedgehogs. He believed that certain physiological processes must be completed before the assumption of active behaviour. This view has been expressed also by Serdjukova (1960) and Kheisin (1954). An interesting point arose from Campbell's experiments in the laboratory. When development occurred at a comparatively high temperature the post-ecdysial changes were retarded at a particular temperature

/in comparison

in comparison to the rate at which they were completed in ticks whose development took place at a lower temperature. The prolonged post-ecdysial period necessary in autumn for completion of the physiological processes could be expected in the conditions of falling temperature at this time of year.

Macleod (1935) found no difficulty in feeding ticks during late autumn and winter, and in experiments here larvae and nymphs emerging in the field in autumn were fed successfully at these times of year. Cameron (1939) found high infestation of adults on deer in midwinter in Perthshire. Campbell (1948) concluded that there was no evidence for the view held by Totze (1933) that unengorged ticks enter a dormant condition of hibernation which is unaffected by the weather conditions during the winter months. On the contrary the evidence, though conflicting, does suggest a long delay in the assumption of active behaviour in autumn; the factors which induce it remain unknown. Alfeev (1948) considered it as an adaption preventing the appearance of active ticks at a time of year when the host potential in forest areas was low. Belozerov (1966) is of the opinion that autumn inactivity is under photoperiodic control. His experiments demonstrating inability to feed in nymphs conditioned under a short photoperiod are not convincing. His youngest population (5 months) shows the best results but the temperature, 18°C, appears to be too high for demonstrating winter or autumn photoperiodic effects. Possibly with lower temperatures and younger ticks an effective result could be obtained.

/The possibility

The possibility that midsummer adult inactivity in Dermacentor species plays a part in the integration of the life cycle with the favourable seasons of the year has already been discussed. No such view has been held for summer inactivity of I. ricinus. Its poor tolerance of low relative humidities (Macleod 1935), its inability to retain moisture and the necessity for recuperative periods in places of high humidity in its surroundings (Lees 1946, Lees and Milne 1951) and its inability to maintain its water balance as its reserves of food become exhausted (Lees 1964) all lend support to Campbell's opinion that spring activity comes to an end with the death of any ticks which have not found a host by the end of June. (Campbell 1948). He was unable after an intensive search of the vegetation mat to find any hungry ticks in mid-summer. In delayed stocking experiments, Milne (1945) was unable to extend appreciably spring activity, and in direct observations on individually marked adults in the absence of the main host Lees and Milne (1951) found few active ticks after mid-July. These observations again tend to support the view that depletion of the unfed population as a result of feeding or death terminates spring activity.

Other observations do not always support this conclusion. Alfeev (1935), in a form of delayed stocking, found that cattle, after the decline in spring activity, became reinfested with ticks when they were grazed on unused forest pastures in mid-summer. Macleod (1939) in field and laboratory observations has correlated field activity with a temperature range between

/45°-60°F and

45°-60°F, and the absence of activity with an avoiding reaction to midsummer temperatures above 60°F. He did not inspect the vegetation mat for inactive but viable ticks. However in Austria where the density of ticks is apparently very high, Loew et al. (1963) state that although many ticks have found a host by the end of spring, the remainder were still present in the habitat but avoided the high temperatures and low humidities prevalent at this time of year, and only became active again in autumn. This might indicate a higher density of ticks in the forest regions of the continent and possibly more favourable conditions for survival. In open heathland in certain areas of South East Scotland and North East England there is no indication of survival or recrudescence of activity in spring of active ticks (Campbell 1948). It is possibly significant that Barnett (1965) records far higher numbers of ticks in the coniferous forests of Thetford Chase (East Anglia) than are found in the more exposed uplands elsewhere in Britain. This was the impression gained by the author from experience of blanket dragging in the two types of habitat.

On the basis of the extensive behavioural studies of Lees (1946, 1948) it appears that in the presence of persistent high temperatures and low humidities all stages of I. ricinus might settle in the higher humidities found below the vegetation.

The adaptive importance of seasonal inactivity in I. ricinus remains obscure. On the other hand the consistent appearance of a delay in development in all stages of the tick

/some time

some time in autumn leaves no doubt that the presence and absence of a developmental diapause is most important in the adaption of the tick to the seasonal fluctuations in their habitat. (Alfeev 1947, Campbell 1948, Babenko 1956 and others.)

Of these authors only Campbell (1948) has attempted an understanding of diapause induction. By incubating engorged field ticks at a range of constant temperatures in the laboratory he found that autumn collected ticks had a much higher temperature requirement for development than spring ticks. At temperatures from 15°- 25°C, the pre-oviposition period, embryonic development and the duration of the mobile phase (diapausing stage of Falke) of larvae and nymphs were prolonged. This diapause (or autumn state) achieved its greatest expression in association with the midsummer experience of the unfed tick in the field. As the autumn season progressed the delays became less prolonged until in spring they had been eliminated. Campbell suggested that diapause was associated with the high temperature experience of the autumn active ticks. The lower temperature experienced by ticks still active in late autumn reduced the diapause intensity, and overwintering experience by all the emerging spring population and any remaining autumn ticks eliminated diapause completely.

Recent experiments have shown that although diapause is associated with the autumn experience of unfed ticks it can be induced directly by the post-feeding photoperiodic conditions. Loew (1964) exposed engorged larvae and nymphs of an Austrian population of I. ricinus to a range of photoperiods at room temperature. Diapause was not observed at 14 hours of light per day and above but occurred at 12 hours and below. Several

/insects

insects have a photoperiodically sensitive diapause stage in which the response is not unlike that of I. ricinus. For example the European corn borer Ostrinia nubilalis can be brought out of diapause by long day conditions (Beck and Alexander 1964).

The present work attempts to assess the relationship between photoperiod and temperature and their role in regulating the life cycle of I. ricinus. Some observations on other Ixodid ticks are also included.

MATERIALS, METHODS AND TERMINOLOGY

Engorged females were collected from sheep and cattle throughout the tick active season in an area of exclusive spring activity (Traquair, Peebleshire, 55°35'N. 3°3'W.) and in another where activity occurs in spring and autumn (Killin, Perthshire, 56°27'N. 4°20'W.).

Adults were never very abundant and immature ticks were not easily obtained from the host. Active nymphs were collected in sufficient numbers from the vegetation using the blanket dragging technique described by Macleod (1932). Thick Turkish towelling proved more successful than blankets; the ticks, once on the towel, crawled rapidly into the thick pile and were not brushed off by farther dragging. When the towel was turned over the ticks soon crawled to the surface again, particularly on warm days, and were readily sucked up into tubes. Larvae were more difficult to collect by this method; suction was less effective in removing them from the towel. They had a higher death rate than the nymphs during and after feeding which farther reduced the size of the samples. Their numbers were supplemented using ticks confined to glass tubes in the vegetation mat, a method adopted successfully by Campbell (1948). Engorged females were collected from sheep and cattle and each confined to 1½" by ½" glass tubes loosely stoppered with non-absorbent cotton wool. The tubes were placed in the vegetation mat with the tops lying downhill to prevent flooding. If spring or autumn larvae were required

/batches

batches from the previous spring or autumn adults were brought into the laboratory for feeding. There were indications that confinement in the tubes may have affected subsequent development (Section III 2) and the life of the spring larvae was certainly extended into July whilst activity ended in June. Presumably confinement limited water loss and conserved resources normally used during activity.

Larvae and nymphs were put onto a laboratory host on the same day or the day after they were collected, and experienced as far as possible only natural conditions in this transition period. Hedgehogs were used for feeding immature ticks; they were collected after engorgement in water trays underneath the cages (Macleod 1932). Rabbits were used in the same way; larvae and nymphs were released and allowed to wander over the host and attach at will. They invariably became too crowded around the eyes particularly, and also at the base of the ears, and around the nose, mouth and chin. Although not within the scope of this work, the behaviour of the ticks on the host is of interest. It was found that ticks were confined to these areas only, whether the rabbits were allowed to scratch or not. If ticks were put on the legs, back, tail or belly their first reaction was to crawl rapidly into the fur; they soon reappeared at the surface and started to crawl against the line of the fur, which inevitably led them to the head region. If a strip of fur was shaved off the back, 10-20% of the larvae hesitated in this area, attached and fed, while the remainder crawled rapidly forward and again attached around the head. Bare

/skin

skin had an attraction for the ticks.

The direction of light (from a window) did not affect their forward movement and disturbance (stroking the fur) caused them to dive down into the fur, only to return shortly to the surface and resume their forward progress. A higher concentration of carbon dioxide around the head was not the attraction of that area. If another rabbit was placed with its nose to the tail of the host, larvae on the host's tail again moved forward. The line of the fur apparently directed larvae, nymphs and adults to the head and ears.

This method of feeding either using hedgehogs or rabbits was too laborious, particularly when many different batches of ticks had to be fed separately and washed two to three times. Instead all stages of the ticks were confined to rabbits' ears in white cloth ear bags and the host restrained by a leather collar.(Plate 1). This method was only slightly adapted from Bailey's technique for feeding Rhipicephalus appendiculatus Neumann, 1901. (Bailey 1960). Although the replete ticks were prone to dessication after dropping from the ears, this usually could be avoided if they were removed from the bags before 11 a.m. There was a daily "dropping rhythm" and most ticks detached between 7 to 11 a.m. If larvae and nymphs were put on to the host on the afternoon of day one, then dropping of the majority of larvae could be anticipated on the morning of day four and of the nymphs on the morning of day five. If light is excluded during feeding (black earbags) the time of dropping was less predictable.

Several other Ixodid ticks were reared successfully for at least one generation using rabbits as hosts. Those

/included

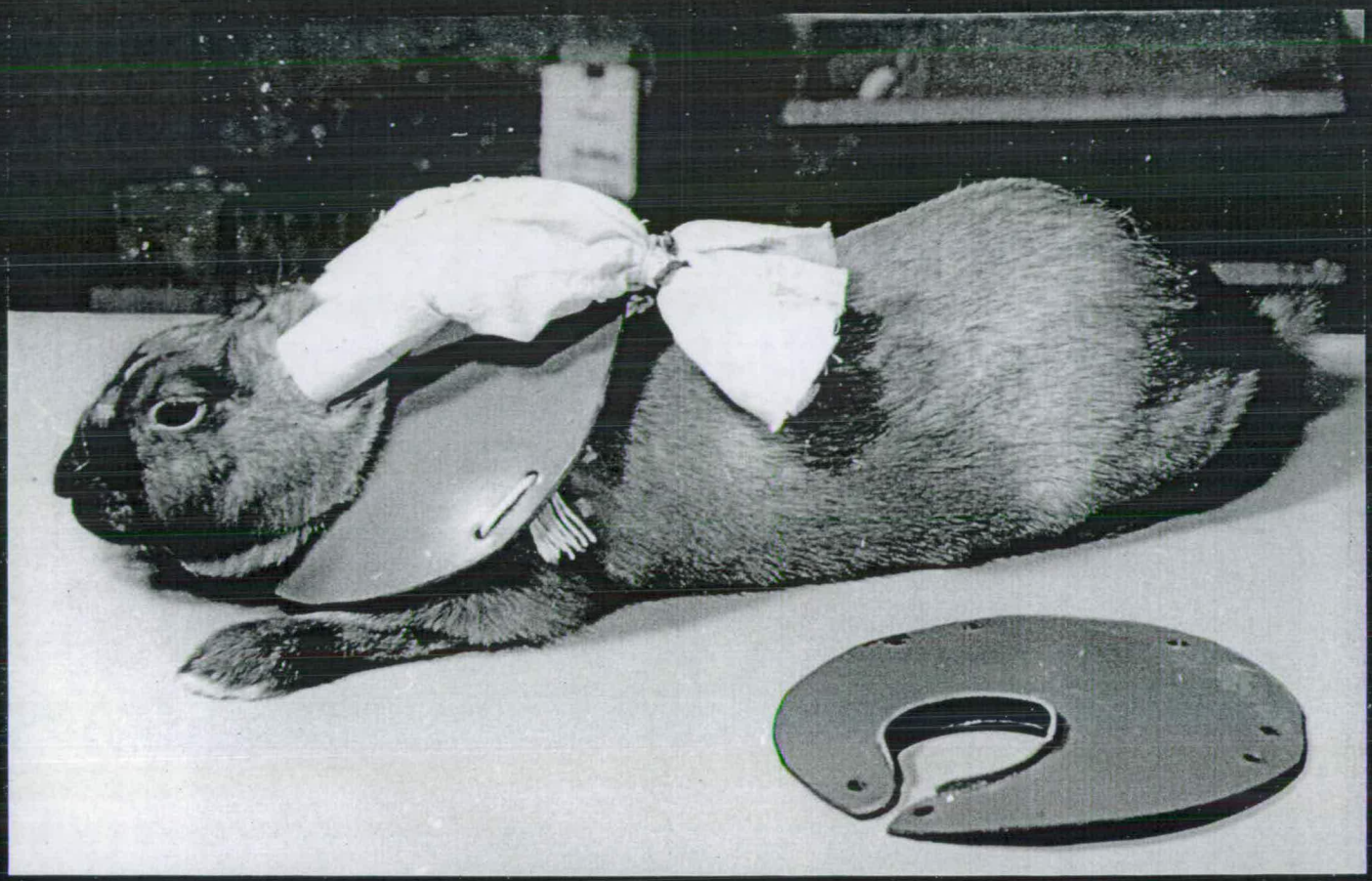


Plate 1. Feeding ticks on rabbits' ears. Collar shown separately.

included Amblyomma gemma Donitz, 1909; Hyalomma anatolicum Koch, 1844; Hy. marginatum, Koch, 1844; Hy. rufipes Koch 1844; Haemaphysalis punctata; H. leporis-palustris (Packard, 1869); Rhipicephalus appendiculatus; R. bursa, Can. and Fanz., 1877; Dermacentor andersoni; Ixodes canisuga Johnston 1849; and I. hexagonus, Leach, 1815. Larvae of Margaropus winthemi Karsch, 1879, attached but failed to feed on a rabbit's ear and Ixodes uriae White, 1852, not unexpectedly failed even to attach. Amblyomma americanum (Linnaeus, 1758) fed successfully up to the adult stage but these never reached full engorgement and laid few viable eggs. All stages of I. canisuga and I. hexagonus, although they became fully engorged, they did so very slowly and were then reluctant to drop from the host. Adults of I. ricinus fed readily on rabbits'ears but many appeared to be feebly attached to the skin and were often knocked off or dropped before completing engorgement.

For feeding the larvae and nymphs of I. ricinus this method had the advantage that two different batches could be fed on the same host and excessive washing avoided. The dried excreta which accumulated during feeding could be sieved off through a small coffee strainer. One disadvantage however, was the resistant reaction of the rabbit. After the third of fourth feeding the skin blistered at the site of attachment and the ticks were frequently engulfed by an oedematous secretion. A similar reaction was described by Trager (1939) in rabbits resistant to D. variabilis.

Engorged ticks were kept in the laboratory in 1½" by ½"

/glass tubes

glass tubes loosely stoppered with non-absorbent cotton wool. Whether field collected or reared in the laboratory not more than one engorged female, twenty nymphs or fifty larvae were kept in each tube. At each incubation, temperature and photoperiod not less than twenty nymphs and thirty-three larvae were used, except on one occasion mentioned in the text. Adults were less abundant and the numbers in each sample are given with each experiment. Inevitably part of a population died after feeding, and if the numbers fell below twenty nymphs or thirty-three larvae before the end of the experiment the results were discarded.

Most of the laboratory observations were carried out at relative humidities near saturation; the small tubes were kept in one quart screwtop glass jars containing a layer of plaster of Paris at the base. The high humidity prevented desiccation of the ticks but encouraged moulds. To prevent this Campbell (1948) recommended washing the ticks in a 0.1% solution of brilliant green or gentian violet. Loew (1964) introduced a cellulose pad soaked in a 5% solution of Nystatin into the tubes and Joyner, Davies and Kendall (1963) mixed charcoal with the plaster of Paris to lower the relative humidity. Various saturated solutions which gave lower humidities (Solutions recommended by Solomon 1951) were used with some success in preventing moulds particularly for species with a lower humidity requirement than I. ricinus. Plaster of Paris was eventually found to be perfectly adequate if the basal layer was initially dried hard and 2 mls. of distilled water

/added monthly

simulate simulate

added monthly. No water condensed inside the tubes and provided tubes containing engorged larvae and nymphs were cleaned some time after feeding and again after the next instar emerged, very few ticks died. Cleaning the tubes was essential whatever method was used, except when ticks were kept at lower relative humidities, but the larvae of I. ricinus did not survive so long at these conditions.

Engorged larvae, nymphs and adults were also kept in the field for comparison with the laboratory observations. They were confined in tubes in the vegetation mat as already described for the engorged females. Only one female, ten nymphs and twenty larvae were put in each tube. High humidities could not be avoided in the field and the ticks were examined and the tubes cleaned in many cases after a long absence. With more ticks in each tube there was a tendency for water of condensation, combined with fluid excreta to swamp the ticks which then stuck together in a wet mass. No ticks developed under these conditions. With fewer ticks per tube these adverse conditions could be avoided, individuals settling in drier corners around the plug.

Although it was intended to compare the field and laboratory results no attempt was made to simulate the fluctuations in temperature found in the field. They are from day to day very variable and for I. ricinus, which survives for many months, there is also a progressive seasonal change. The temperature experience of the fed ticks is different from the active ones whose time is divided between the base and the tips of the

/vegetation

vegetation (Lees and Milne 1951). Instead, a range of temperatures were established in the laboratory, these included 2-5°, 10°, 15°, 18.5° and 23°C. Species with a higher natural temperature experience than I. ricinus were incubated at 10°, 15°, 18.5°, 23° and 25°C. The lowest temperature, 2-5°C. was maintained in a refrigerator, the bio-kinetic temperatures within the natural experience of I. ricinus, 10°-18.5°C. were maintained in a cold room. The room itself was kept at 10°C., just above the lower limiting temperatures for the different developmental stages of the tick. Standard incubators were kept in this room and their bimetallic thermo-stats adapted to give temperature control at 15°C. and 18.5°C., at or just above the mean midsummer maximum in the tick habitat (Campbell 1948). Higher temperatures, 23° and 25°C. in thermostatically controlled insect rooms were used on occasions.

The experimental conditions in the cold room (Plate 2) were not entirely satisfactory, as the room itself was subject to mechanical, electrical and human faults, and as there was no auxiliary motor, results had occasionally to be discarded. The incubators though giving satisfactory temperature conditions were usually 1°C. warmer when the lights were on despite the thick glass door between the bulb and the interior. Incubators with long photoperiods were set slightly lower to compensate for this deficiency.

The temperature and photoperiodic conditions which were eventually established are summarised below. The maximum fluctuation in the temperatures were taken from many

/observations

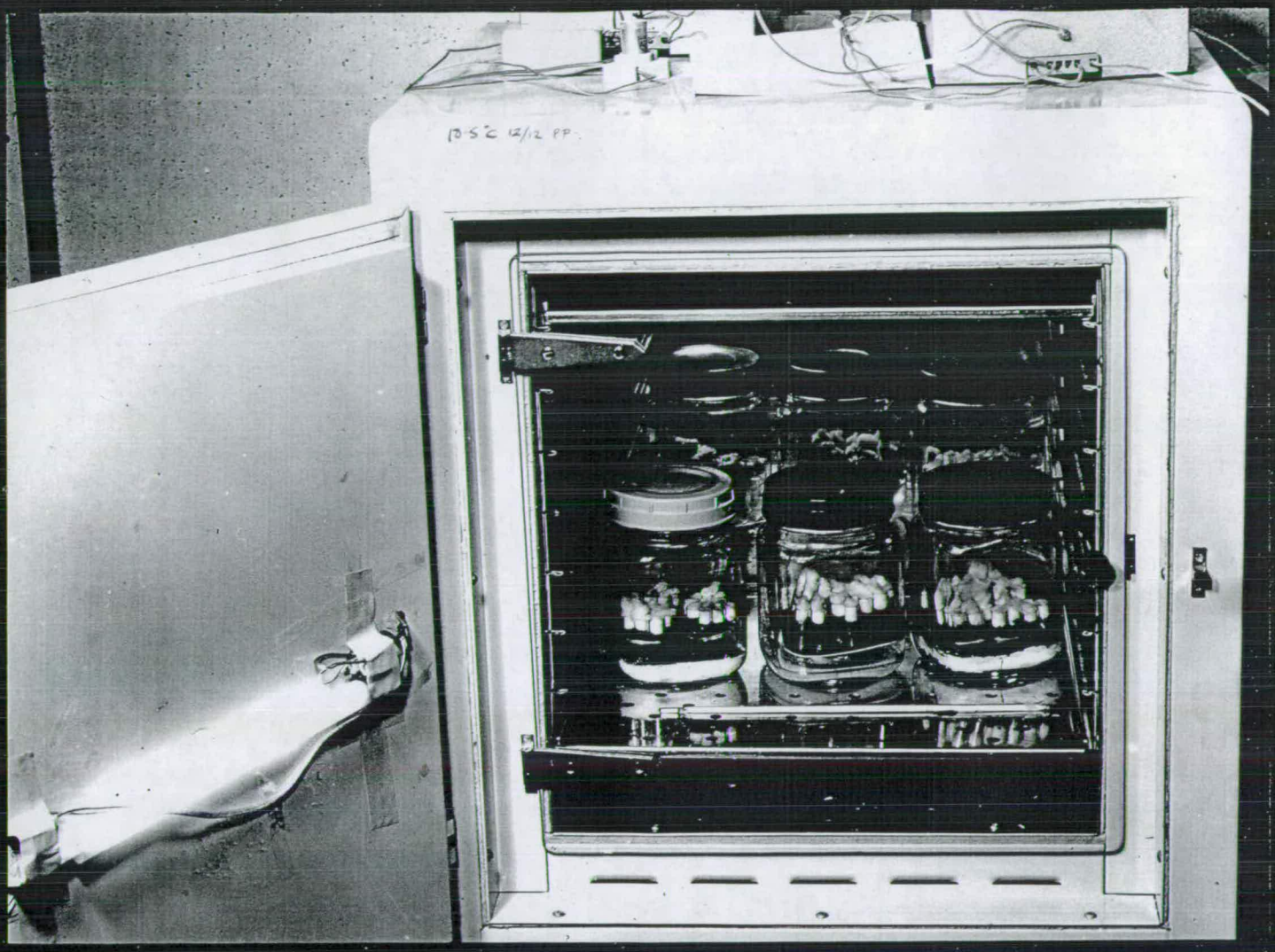


Plate II. Incubator containing tick jars. A thick glass plate separates the interior from the light source.

observations during the light and dark periods while ticks were being incubated.

Temperatures °C. 25±3.0; 23±2.5 23±2.0; 18.5±1.9 18.5±1.7;
Light-dark cycles 18:6 ; 18:6 10:14 ; 18.6 12:12 ;

Temperatures °C. 15±2.1 15±1.2 15±1.6 15±1.4; 10±1.8 10±2.1;
Light-dark cycles 18:6 16:8 14:10 6:18 ; 18:6 14:10 ;
18:6 and 0:24 at 2-5°C; and solar photo-periods at 15±1.0°C.

While the swing in some cases was quite large, the incubators at 10°, 15°, and 18.5°C., from which most of the results have been taken, were checked almost every day during the experiments and any deviations rapidly corrected.

The photoperiodic conditions had to be chosen with some care; once established they could not be changed over a 2½ years observation period on field and laboratory ticks. There was a limit to the number of permutations of temperature and daylength which could be checked at sufficiently frequent intervals.

In assessing the ecological significance of the photoperiodic control of diapause, the critical value is of primary importance. Thus diapause in engorged larvae and nymphs of an Austrian population of I. ricinus was prevented at 14 hours of light per day but induced at 12 hours per day (Loew 1964). On the assumption that the critical value for a Scottish population would be longer, between 16 and 14 hours of light per day, these two photoperiods were established. The 18 and

/6 hour

6 hour photoperiods represent the longest and shortest days naturally experienced by fed or unfed ticks. The solar photoperiod changed daily in synchrony with the daylength (sunrise to sunset) at a latitude 57°0' North, longitude 3°45' West. This was slightly farther North than the actual localities visited, but as the intensity of light at dawn and dusk may have been above the threshold for perception there was little point in attempting an accurate correlation between the field and laboratory regimes. The annual photoperiodic rhythm is given in Sect. IV, 4.

Only a long and a short photoperiod or darkness were used at temperatures other than 15°C.; the object was to find in what way if any the temperature modified the photoperiodic response in I. ricinus and to provide conditions nearer to that experienced in the field by such species as D. andersoni, H. punctata and R. bursa.

The duration of the light and dark periods was controlled by Londex or Venner time switches; these were checked during the experiments, and did not vary by more than 7 minutes from the times given.

It was important that the light intensity in the incubators should be above the threshold recognised by the tick. In I. ricinus, as in other arthropods, this is relatively low; Loew (1964) obtained the typical long and short day response at an intensity of 5 lux. The incubators were fitted with Philips No. 33 (Cool white), 12 inch, 8 Watt fluorescent bulbs. Their average output through life was 335 lumens. In other

/words

words an object one metre from the bulb would receive an intensity of 335 lux. As the ticks were within 40 cms. of the source, and light was reflected from all surfaces of the incubator, this type of bulb was considered suitable. Some doubt still remained as to whether the intensity of light was sufficient to penetrate the dense larval masses which congregated at the tops of the tubes. As a control, weekly egg batches from engorged autumn ticks were kept in longer tubes (2½" by ½"), once the larvae emerged the tubes were inverted every two days for eight weeks. The ticks were not allowed to settle in a dense mass but were continually crawling or at rest fully exposed to the light. Their response to long and short days differed in no way from larvae which were not separated into smaller batches and were allowed to aggregate at the top of the tubes.

Another important point is the sensitivity to different wavelengths of light. This is not known for I. ricinus, but the bulbs used had a wide spectral range 4000-7000 Å, covering wavelengths in the blue and blue-green which are most active photoperiodically in several Arthropods (Lees 1955, Danilevsky 1965).

The purpose of keeping ticks in the laboratory was primarily to determine the characteristics of development associated with different pre-feeding experience. Because of the unusual sensitivity of the diapausing stage this has not been easy and it is necessary to define clearly the stages of development and the terms used, some of which have been

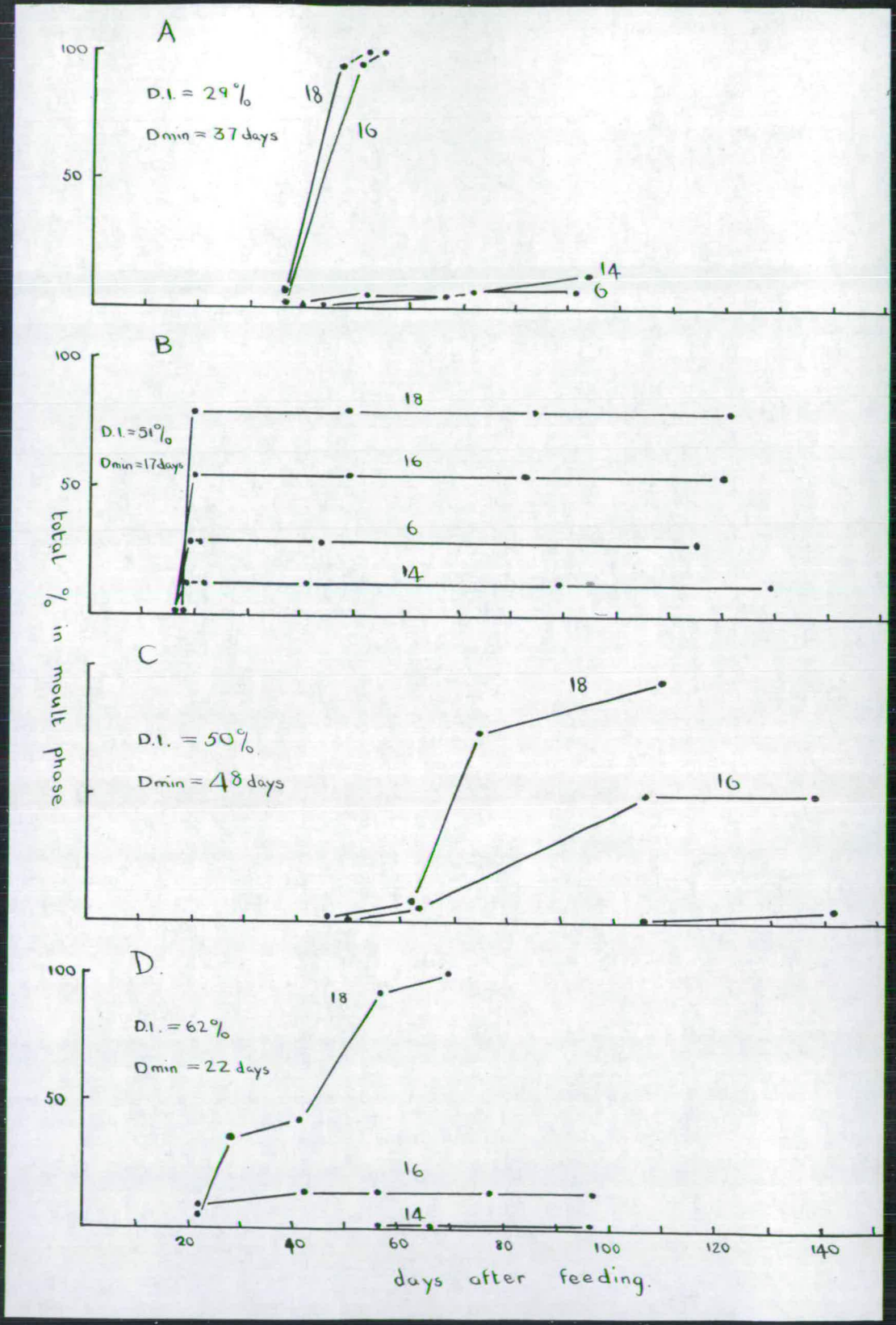
/adapted

adapted to suit the occasion.

Development of engorged immature ticks is divided into two visibly distinct stages. At first the ticks remain mobile and respond to external stimuli but then take up a characteristic posture with legs outstretched. In this position they very rapidly lose mobility and after some time the next instar emerges. Falke (1931) recognised that diapause was a prolongation of the mobile phase but unfortunately called it the Vorruhestadium or Pre-resting stage. Campbell (1948) preferred to use the term pre-developmental stage, but this is also ambiguous, development of certain tissues taking place at this time (Bonnet 1907). The term pre-moult phase (or the pre-moult) has been used here for the mobile stage and moult phase for the period starting with immobility or separation of the epidermis from the cuticle and ending with ~~the~~ eclosion.

Diapause in I. ricinus can be regulated by the direct action of temperature and light on the pre-moult phase, but the response to these external stimuli is very variable. For example, if a population of engorged ticks were incubated at 15°C. all entered the moult phase at long photoperiods but almost all were in diapause at short ones (Graph A). There was a reasonably well defined critical photoperiod between 14 and 16 hours of light per day. In another population photoperiod had less significance for the population as a whole, individuals diapausing at 18, 16 or 14 hours of light per day (Graph B). In some populations there was a prolonged

/pre-moult



Photoperiodic response at 15°C of different populations of I. ricinus. The numbers in each graph (18, 16, 14 and 6) indicate the hours of light per day. D.I. is the Diapause incidence, D. min. the minimum duration of the pre-moult phase at 15°C LD 18:6.

pre-moult phase (Graph C) or prolonged in some of the ticks but not in others (Graph D). When comparing the different responses of these populations no one criterion is sufficient. The term critical photoperiod has not been used as it is ill-defined in many cases, but if the majority of ticks develop at one photoperiod but are in diapause at another then the threshold photoperiod is considered to lie between these two. In A, B and C the threshold was between 16 and 14 hours of light per day and in D between 18 and 16.

The rise in threshold in Graph D indicates a higher diapause incidence (D.I.) in the population, this is taken as the total percentage in diapause at 18, 16 and 14 hours of light per day. Difficulty in deciding when to determine the diapause incidence was sometimes experienced particularly when development to the moult phase was protracted (Graph C). In most experiments the ticks which are eventually to enter the moult phase will all have done so by 150 days incubation at 15°C. If a tick is still in the pre-moult at this time it is taken to be in absolute diapause, and in a population where the pre-moult is very long diapause incidence and threshold photoperiod are recorded after 150 days. A time limit was necessary here; after about 200 days spontaneous recovery from diapause occurred in many cases , and this was quite unrelated tc the earlier characteristics of development.

To distinguish between groups with prolonged development (Graph C) and ones with a brief pre-moult (Graph B) the minimum duration of the pre-moult phase (D. Min.) is given on

/the graphs

the graphs. Only the duration for ticks at the longest photoperiod has been considered, although the minimum duration at the other photoperiods was usually of the same order. L.D. is the usual convention for the light-dark cycles in a 24 hour day.

These terms were useful, but when presented in a tabular form did not make comparisons between experiments particularly easy. Instead the complete graphs were given for each experiment together with the incidence of diapause and minimum duration of the pre-moult.

Handling of the engorged ticks was performed as follows. After feeding any dead or wrinkled individuals were discarded and the rest kept in the small glass tubes. They were disturbed as little as possible during the pre-moult. With experience a rough estimate of the minimum duration was possible and the first counts were not made until just before this time. Observations were made only when the incubator lights were on. Ticks which were incubated at 15°C. were taken to the laboratory and the jars immersed in cold water at a temperature near 15°C. Tap water even in midsummer never rose more than a degree above this temperature. Tick tubes were removed to a room temperature between 17-21°C. for a short time, sufficient to remove the ticks with a camel hair brush, count the number in the moult and pre-moult phases and the number dead, wipe out the tube with absorbent cotton wool if necessary and return the ticks to the tubes and then back to 15°C. Ticks incubated at 10°C. were counted in the cold room and those at 18.5°C., 23°C.,

/and 25°C.

were counted in rooms at 17-21°C, 23° and 25°C. respectively.

Dead ticks at the first count could be attributed to dessication or damage during or after feeding and these were ignored unless the number of viable ticks fell below twenty nymphs or thirty-three larvae, in which case the experiment was abandoned: once over the initial period of incubation, death rate wa s low and dead ticks were included in the numbers still in the pre-moult phase. If all the mobile ticks died before completion of the experiment a cross (+) was marked on the graph. The way in which the data has been used reduced the discrepancies through death in the population to negligible proportions.

Counts were made initially every two or three days, but as the results became clear the inspection period gradually increased until after 100 days the tubes were examined every two to three weeks. Although the time when the next instar emerged was also noted in some experiments, there were disadvantages in using the whole period from feeding to eclosion as an indicator of diapause, despite the extensive use of this criterion in the literature (Nuttall et al., 1911, Campbell 1948, Kheisin et al., 1954a. and others). Not uncommonly ticks in the moult phase fail to emerge, particularly at a low temperature (10°C.). The moult phase in many populations is longer than the pre-moult and although little affected by the presence or absence of diapause there was a greater dispersion in the time from feeding to eclosion than from feeding to the beginning of the moult phase. This tended to obscure the temporal

/relations

relations of the diapausing pre-moult stage.

Embryonic diapause can not be confined on the basis of macroscopic observations to a particular stage of development. The method adopted by Campbell (1948) has been used here, a prolongation in the period from the beginning of oviposition to the emergence of the first larvae in each egg batch was taken as an indication of diapause. Attempts to observe development of individual eggs in batches of 30-40 placed on damp filter paper were frustrated by the high death rate resulting from even the gentlest separation of the eggs from the main egg mass. This difficulty was encountered by both Macleod (1935b) and Campbell (1948). In some experiments weekly egg batches were isolated by removing the engorged females to fresh tubes, and the appearance of the first larvae in each weekly batch confirmed the importance of daylength for development.

The following section is concerned with the temperature and photoperiodic relations of the engorged field ticks. The influence of these two factors on the fed and the unfed ticks reared in the laboratory is then considered, and their possible roles in regulating the life cycle discussed. Observations on development and activity in the field and in outdoor conditions farther emphasise the well integrated seasonal cycle in I. ricinus.

Some comparable information on photoperiodic control of diapause is given for other Ixodid species.

III

THE SEASONAL DEPENDENCE OF DEVELOPMENT IN FIELD COLLECTED TICKS.

1. The seasonal rhythm in response to daylength in engorged larvae and nymphs.

The response to daylength in ticks collected in 1965 changed with the different seasonal experience of the active stages. This was not simply the appearance of diapause in autumn and its absence in spring but rather a seasonal rhythm in the intensity of diapause, with a peak in July and August and a trough in May. This has also been the experience of other authors (Totze, 1933; Kheisin, 1954;). In 1966 larvae and nymphs were collected more frequently; the larvae were mostly from tubed ticks, samples being taken when required.

Larvae from spring laid eggs began to emerge in Autumn. The first sample was taken on the 14th October, 1965, although activity in the field would not usually be expected until the following spring. Samples were taken up to the 31st July, 1966, by which time field activity had already ceased and most of the confined larvae were dead. The influence of photoperiod on development of this spring series of larvae incubated at 15°C. is given in figure 1.

Diapause incidence was high when larvae were fed in autumn, soon after emergence (44%). Incidence was lowest in spring (11-12%) and a 14 hour photoperiod above the threshold for the first time. By the beginning of July incidence was again higher (24%) and at the end of the month only half the population entered the moult phase at the longest photoperiod

/(LD 18:6).

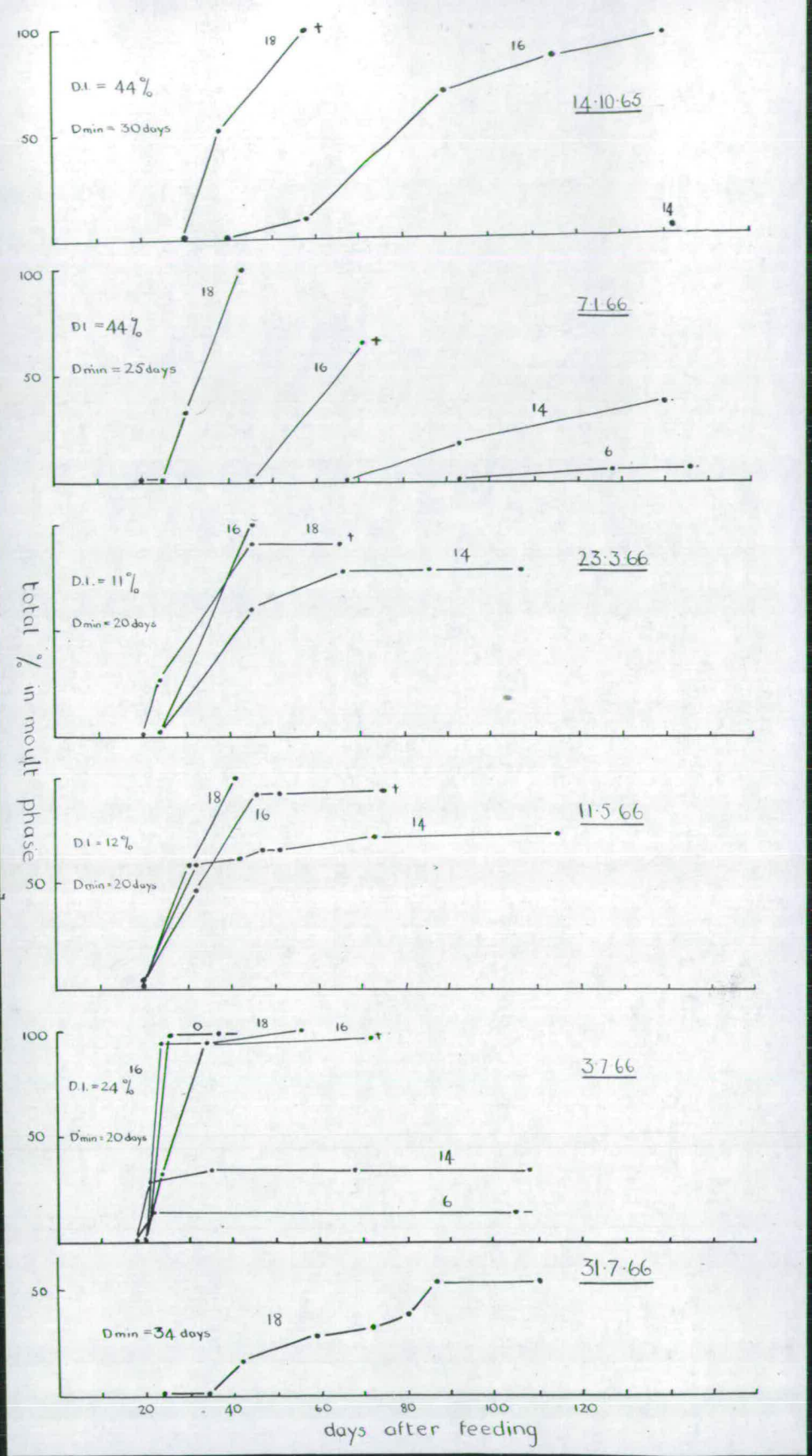


Figure 1. Development of Perthshire larvae at 15°C. The ticks were taken from the field between the 14th October, 1965 and the 31st July, 1966.

(LD 18:6). (Only 20 larvae in this sample)

The few larval samples of sufficient size which were collected by blanket dragging in 1965 and 1966 are included in Table 1.

Table 1. Response to daylength in larvae collected by blanket dragging in 1965 and 1966, and incubated at 15°C.

ORIGIN AND DATE COLLECTED	NUMBER	TOTAL % IN MOULT PHASE AFTER 150 DAYS AT 15°C AND; LD 18:6	LD 16:8	LD 14:10	DIAPAUSE INCIDENCE	MINIMUM DURATION OF THE PRE-MOULT IN DAYS
Peebleshire, 6th Apr. 1965	75	100	-	34	-	-
Perthshire, 12th May 1965	30	100	100	96	1.3	-
Perthshire, 4th June 1965	18	100	100	90	3.3	6
Perthshire, 27th Sept. 1965	45	100	10	0	63.3	-
Peebleshire, 12th Apr. 1966	20	100	-	-	-	23
Peebleshire, 30th Apr. 1966	70	100	100	68	10.7	20
Peebleshire, 13th May 1966	32	100	100	81	6.3	19
Peebleshire, 6th June 1966	22	100	-	72	-	19

These results confirmed the low diapause incidence in the spring active period from April to June.

At an incubation temperature of 15°C. , diapause incidence was always higher in the nymphs, and there was again a seasonal rhythm. In the Peebleshire populations (Figure 2) incidence and the threshold photoperiod were lowest in May. Diapause incidence fell from 26.6% in March to 18.7% on the 26th May, but in the eleven days from the 26th May to the 6th June it rose

/sharply

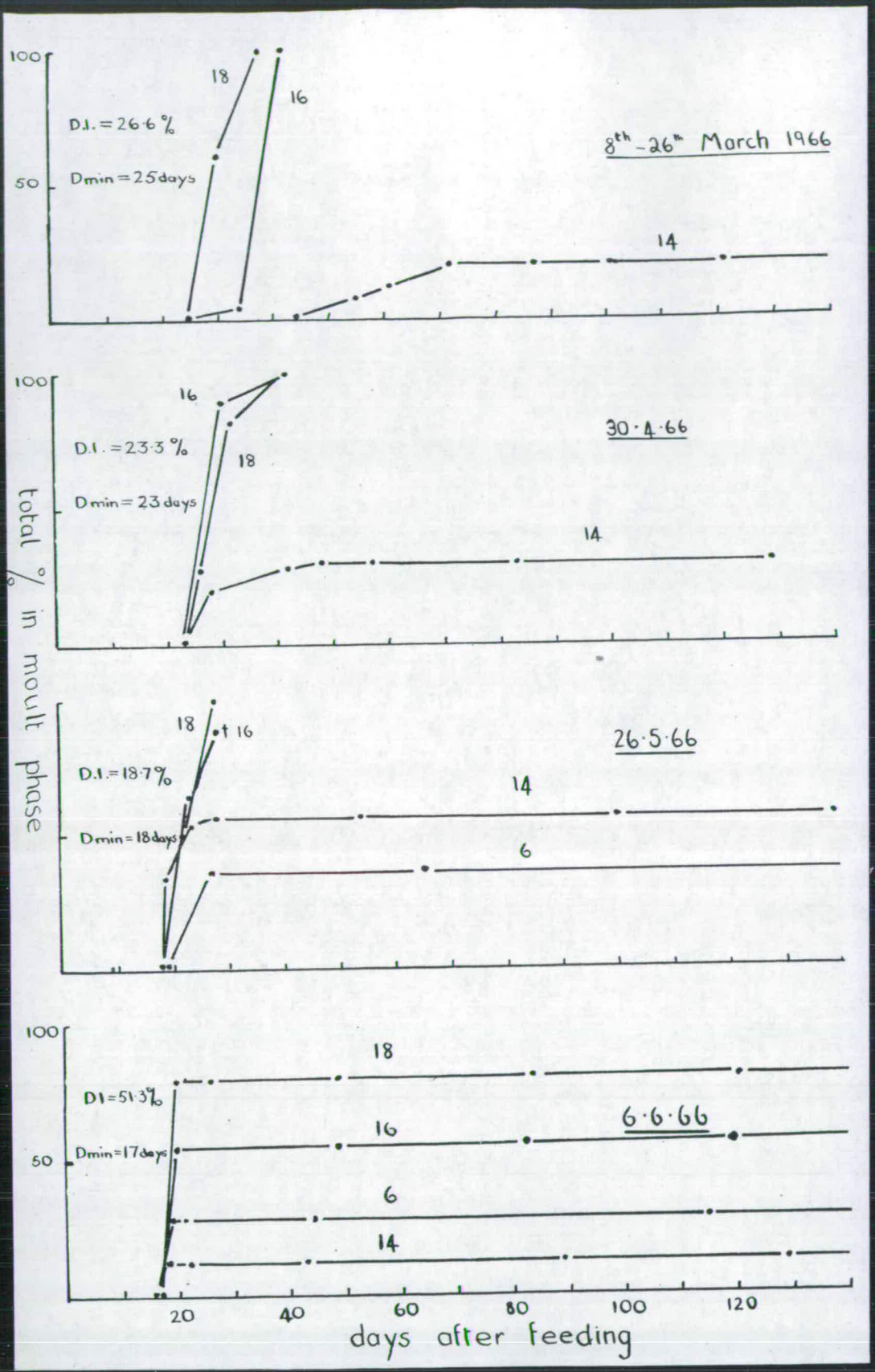


Figure 2. Development at 15°C of Peebleshire nymphs collected between the 8th March and 6th June, 1966.

sharply to 51.3% and even at the longest photoperiod (LD 18:6) 20% were in absolute diapause.

The Perthshire field collections were supplemented with tubed ticks in November and July when few or none were active. For the July sample nymphs collected by dragging on the 28th May were kept in the field until feeding on the 19th July (Figure 3.) Spring nymphs fed in the same autumn that they emerged had a high incidence of diapause, 60%. By May the incidence had fallen to 23.3% but between the 28th May and the 12th June it rose again from 32% to 87%. A total of 76% were in absolute diapause at the longest photoperiod (LD 18:6). Of the nymphs experimentally delayed in the field until July, 97% were in absolute diapause in long days (LD 18:6). The threshold photoperiod fell to between 14 and 16 hours of light per day in May but rose again with the increasing diapause incidence in June.

The rather scanty data for 1965 (Table 2) confirmed the appearance in June of diapausing individuals at optimal light conditions and also confirmed the lowest incidence in May.

Table 2. The response to daylength in engorged nymphs collected in 1965 and incubated at 15°C.

ORIGIN AND DATE COLLECTED	NUMBER	TOTAL % IN MOULT PHASE AFTER 150 DAYS AT 15°C. LD 18:6	LD 16:8	LD 14:10	LD 6:18
Peebleshire,					
31st March	20	100	-	0	20
6th April	34	100	-	3	23
31st May	21	100	-	60	85
Perthshire,					
1st April	23	100	-	0	11
12th May	25	100	100	86	66
4th June	25	76	-	4	4

/The changing

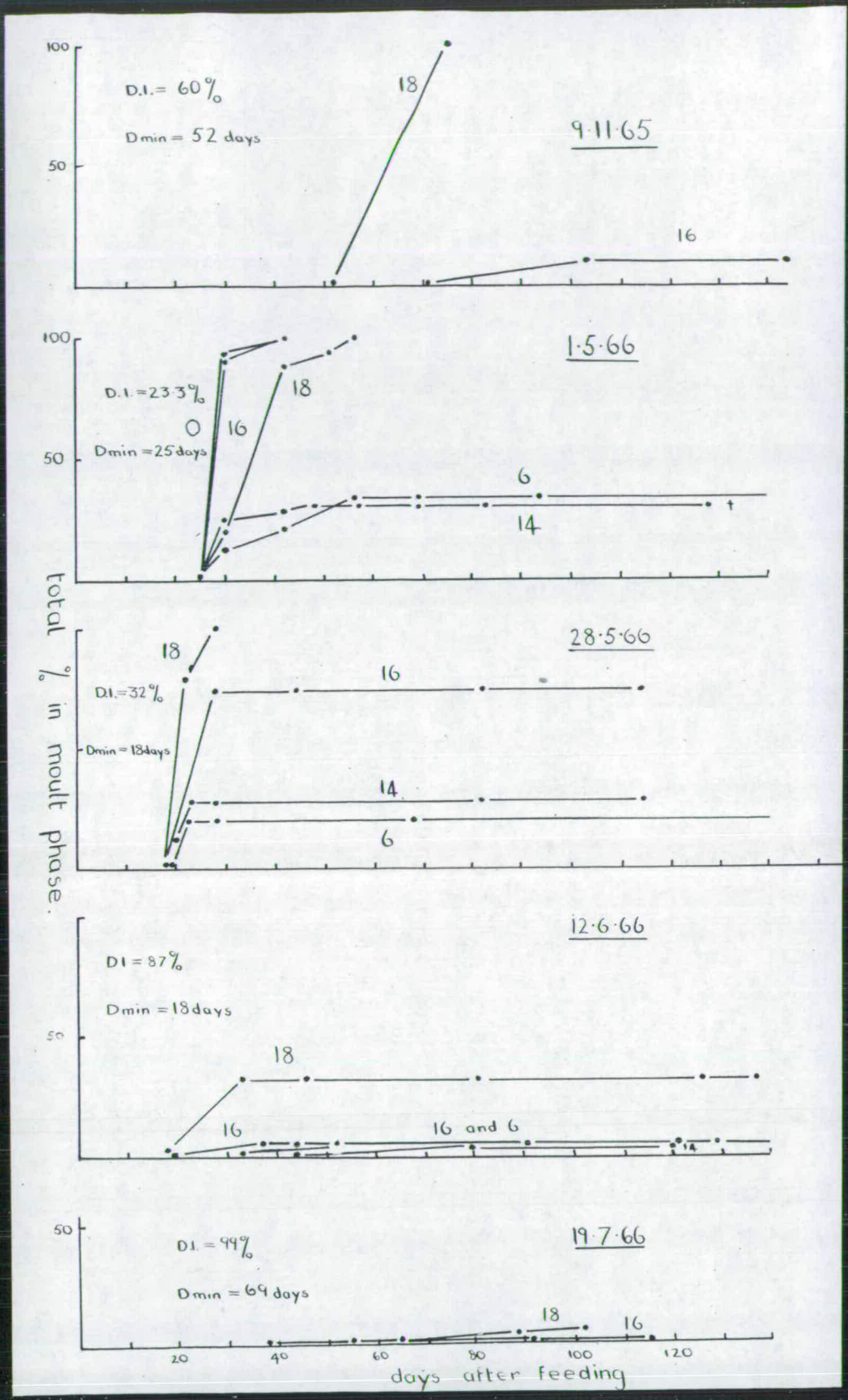


Figure 3. Development at 15°C of Perthshire 'spring' nymphs collected between the 9th November, 1965 and the 19th July, 1966. The first and last samples were 'tubed' ticks.

The changing photoperiodic relations of spring larvae and nymphs are summarised in Figure 4. At a temperature of 15°C. larvae fed during the spring active season developed with little regard to the photoperiod, only when feeding was experimentally delayed until July was diapause induced, and then only at short photoperiods. Nymphs at this temperature were particularly sensitive to photoperiod when fed in early spring. By June and July the photoperiodic effect was masked by an intense diapause at all daylengths.

In autumn diapause incidence was highest among the earliest larvae fed in August (62%)(Figure 5). These first larvae began to emerge from autumn laid eggs at the end of June 1966 and were fed on the 2nd August. Their pre-feeding history was a little different from the other samples in which larvae began to emerge only at the end of July. Diapause incidence was a little lower in these larvae but not strikingly different from September to November (41-50%).

There was a very noticeable change in the photoperiodic relations of engorged ticks associated with the pre-feeding experience during spring and autumn. A typical autumn population (collected 2nd September 1966) is compared with the spring larvae for the same year (Figure 4, broken line, Graph A). Even at a 16 hour photoperiod at 15°C., absolute diapause was not prevented in some of the autumn ticks, and in August this photoperiod was below the threshold for the majority. The only autumn larvae collected by blanket dragging showed clearly the rise in threshold in autumn.(Table 1, page 30).

/When feeding

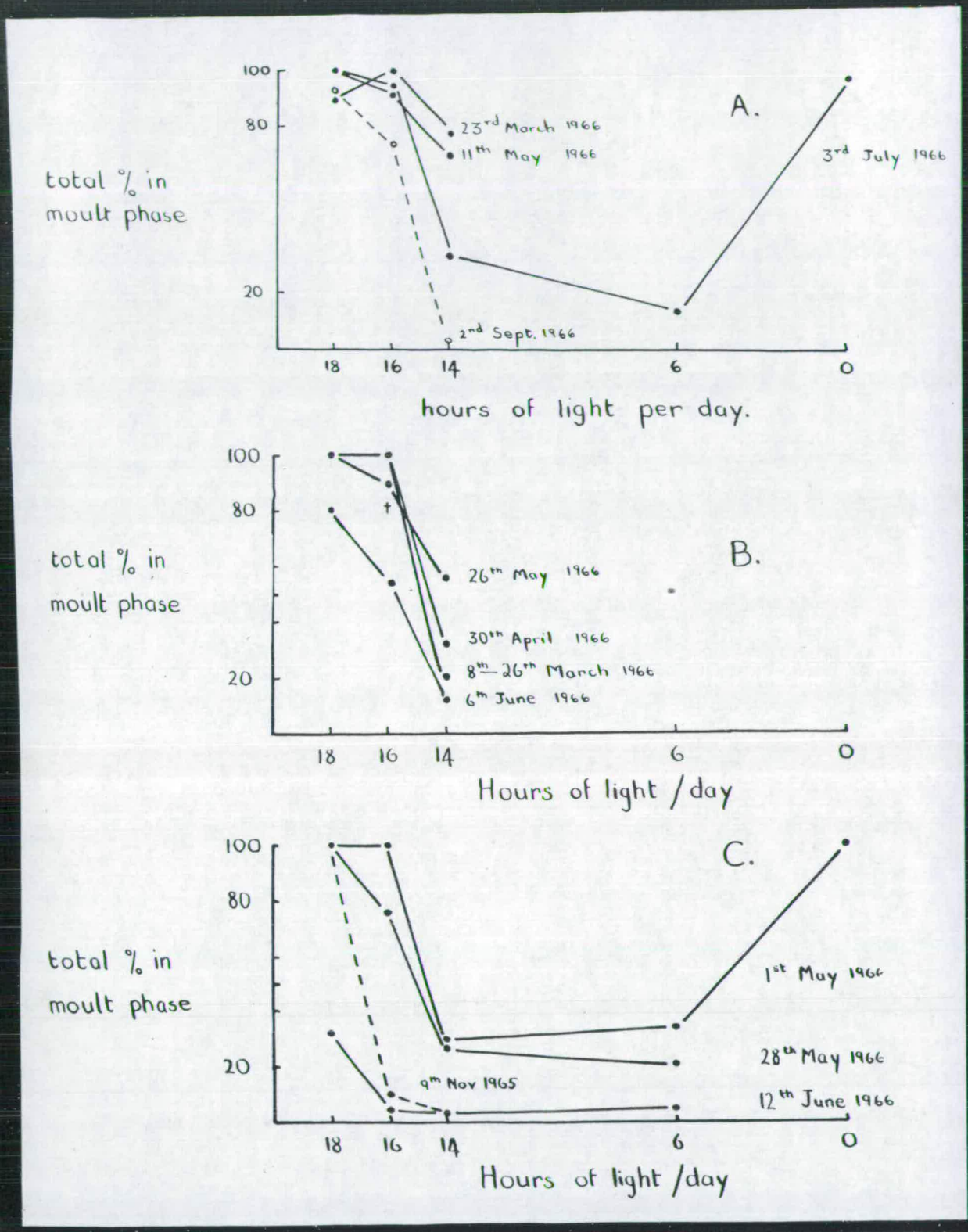


Figure 4. Changes around the threshold photoperiod at 15°C.
A. Perthshire larvae collected in 1966. The broken line graph shows the threshold for a typical autumn population.
B. Peebleshire 'spring' nymphs collected in 1966.
C. Perthshire 'spring' nymphs collected in 1966.

The broken line graph shows the threshold for 'tubed' ticks.

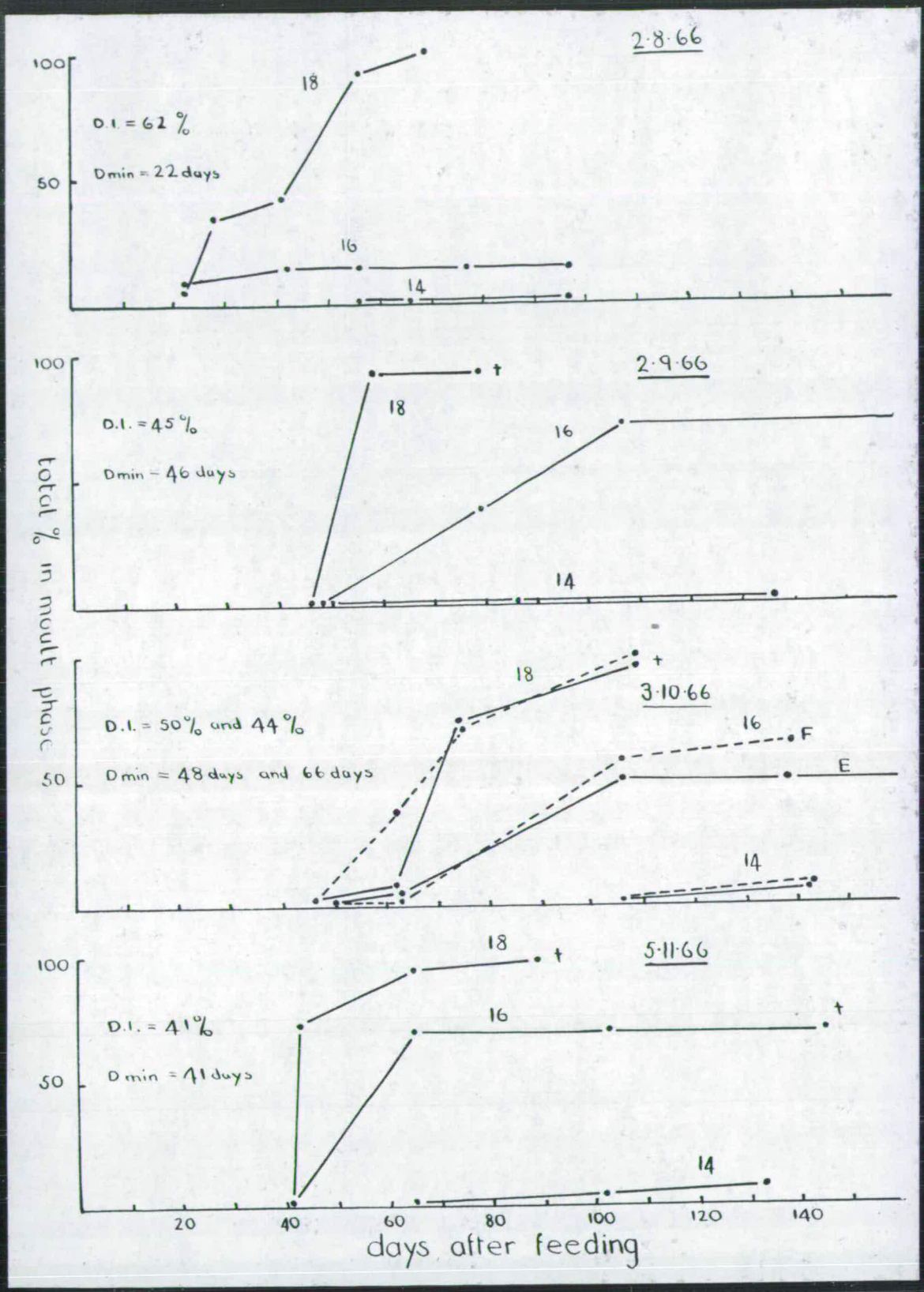


Figure 5. Developmenh at 15°C of Perthshire autumn larvae collected between the 2nd August 1966 and the 5th November, 1966.

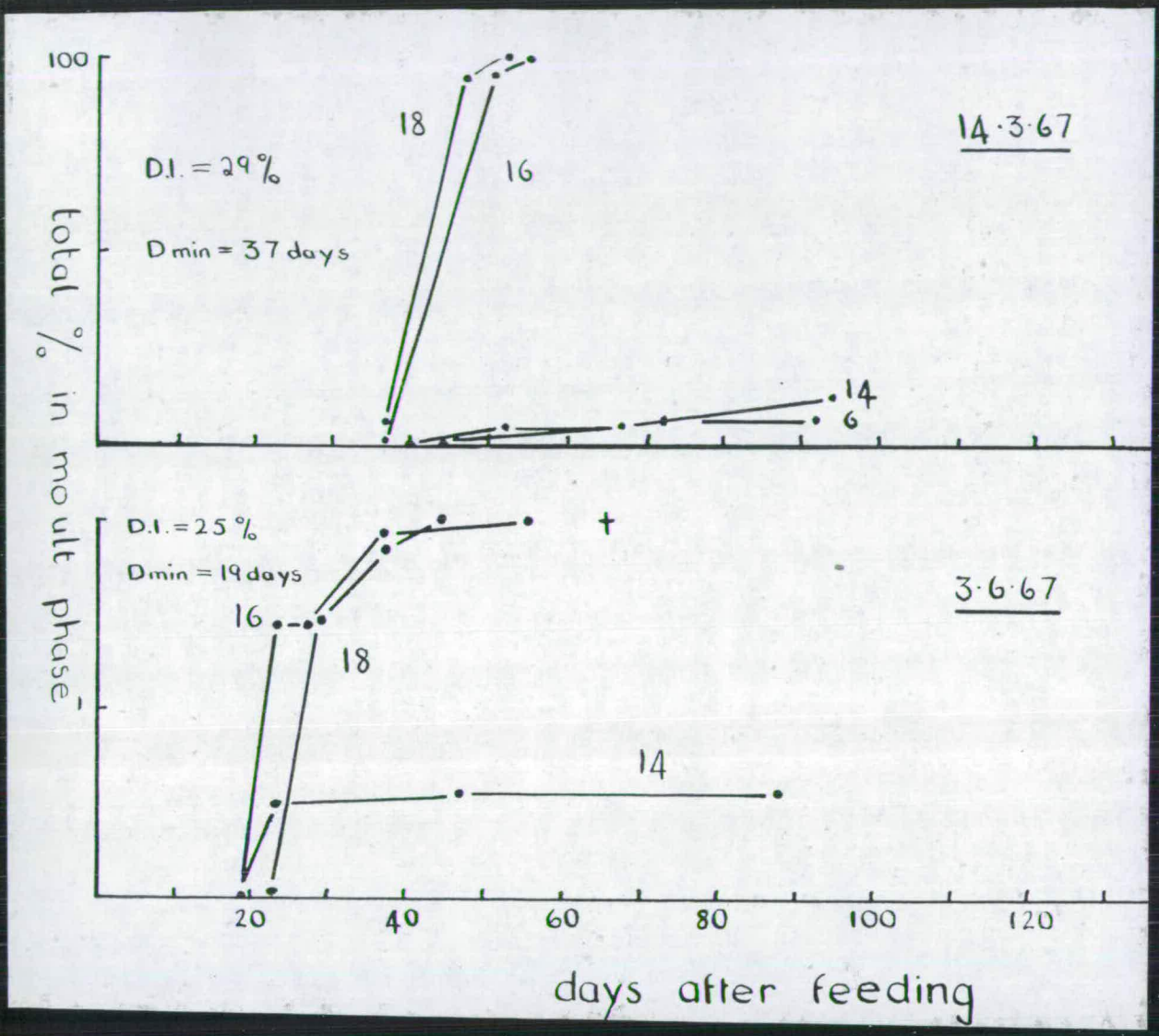


Figure 5 continued. 'Autumn' larvae delayed in the field overwinter before feeding.

When feeding of these autumn larvae was experimentally delayed until the following spring diapause incidence was lower particularly among larvae delayed until June (25%) (Figure 5).

A normal occurrence in the field is the wintering of engorged autumn females before the eggs are laid. No difference was found in the development of autumn larvae derived from ticks which experienced winter conditions as engorged females or as eggs (Figure 5, broken line graph). Only the October populations were compared.

In autumn, diapause incidence was very high in the nymphs incubated at 15°C. Absolute diapause was only prevented at the longest photoperiod (LD 18:6) and the pre-moult for these nymphs was very protracted (Minimum 44 to 150 days or more). Diapause incidence was highest in August (100%) and slightly lower in late September (85%) (Figure 6).

The overwintering experience of autumn nymphs which were experimentally delayed in the field until spring lowered the diapause incidence from 85% to 29.7%. The last graph in Figure 6 compares the development of October 1965 nymphs with the same population delayed until March 1966.

A low but consistent reduction in diapause incidence is found within the autumn active season. In 1965 it was lower in September and October than in July (Table 3).

Table 3. The response to daylength in nymphs collected in autumn 1965 and incubated at 15°C.

DATE COLLECTED	NUMBER	TOTAL % IN THE MOULT PHASE AFTER 150 DAYS AT 15°C.			
		LD 18:6.	LD 16:8.	LD 14:10.	DIAPAUSE INCIDENCE %
25th July	22	5	0	0	98.3
27th Sept.	25	50	0	0	83.3
17th Oct.	21	45	0	0	85.0

/The combined

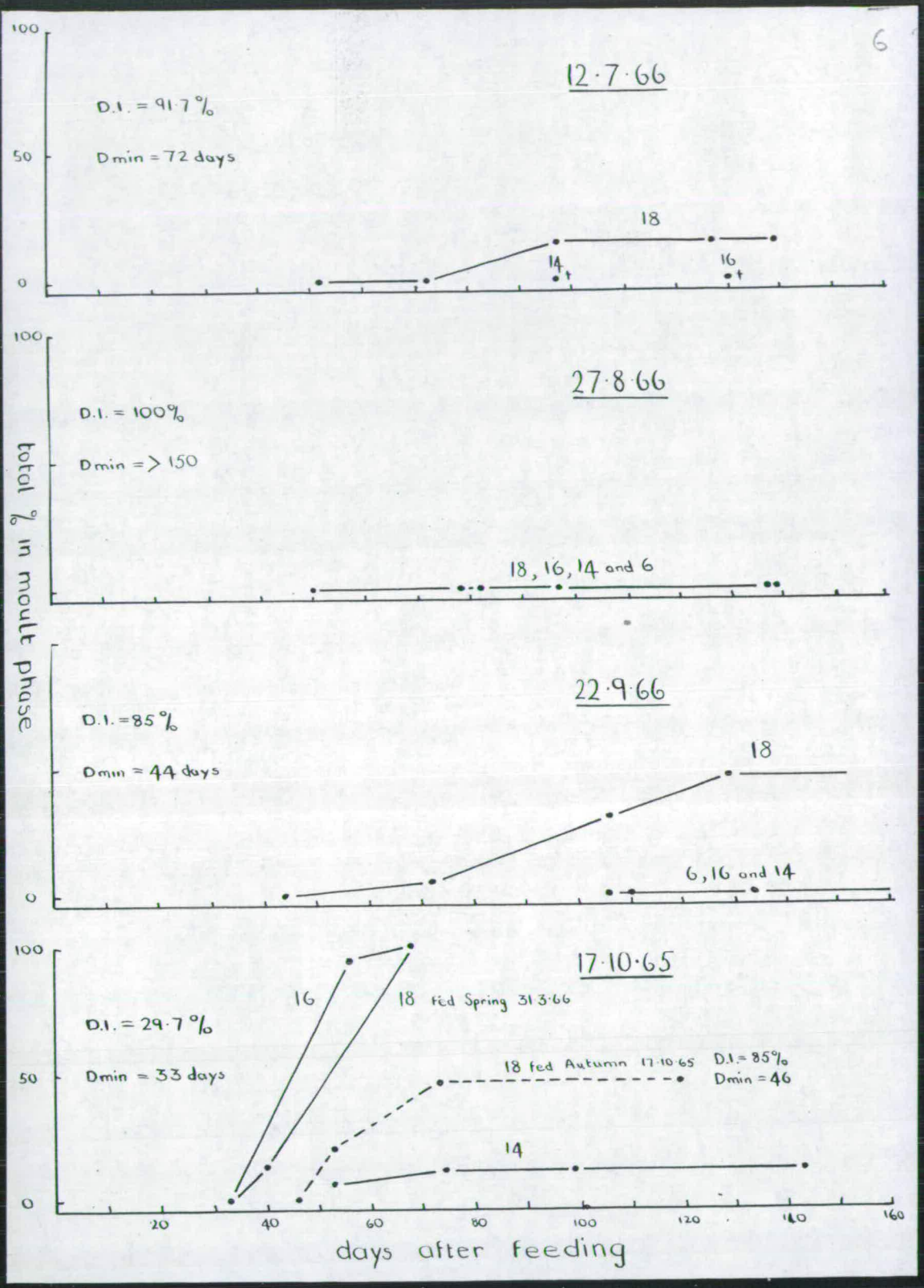


Figure 6. Development at 15°C of Perthshire autumn nymphs collected between the 12th July and the 22nd September, 1966. The last graph shows the development of and'autumn' population delayed in the field overwinter before feeding. The broken line graph shows the development of the original autumn population.

The combined observations on larvae and nymphs were too scanty to confirm Campbell's view that there is a progressive decline in the diapause phenomena with the advance of the autumn season (Campbell 1948). The incidence of diapause at 15°C. was certainly lower in the later months and after experience of winter conditions.

2. Seasonal changes in the duration of the pre-moult phase

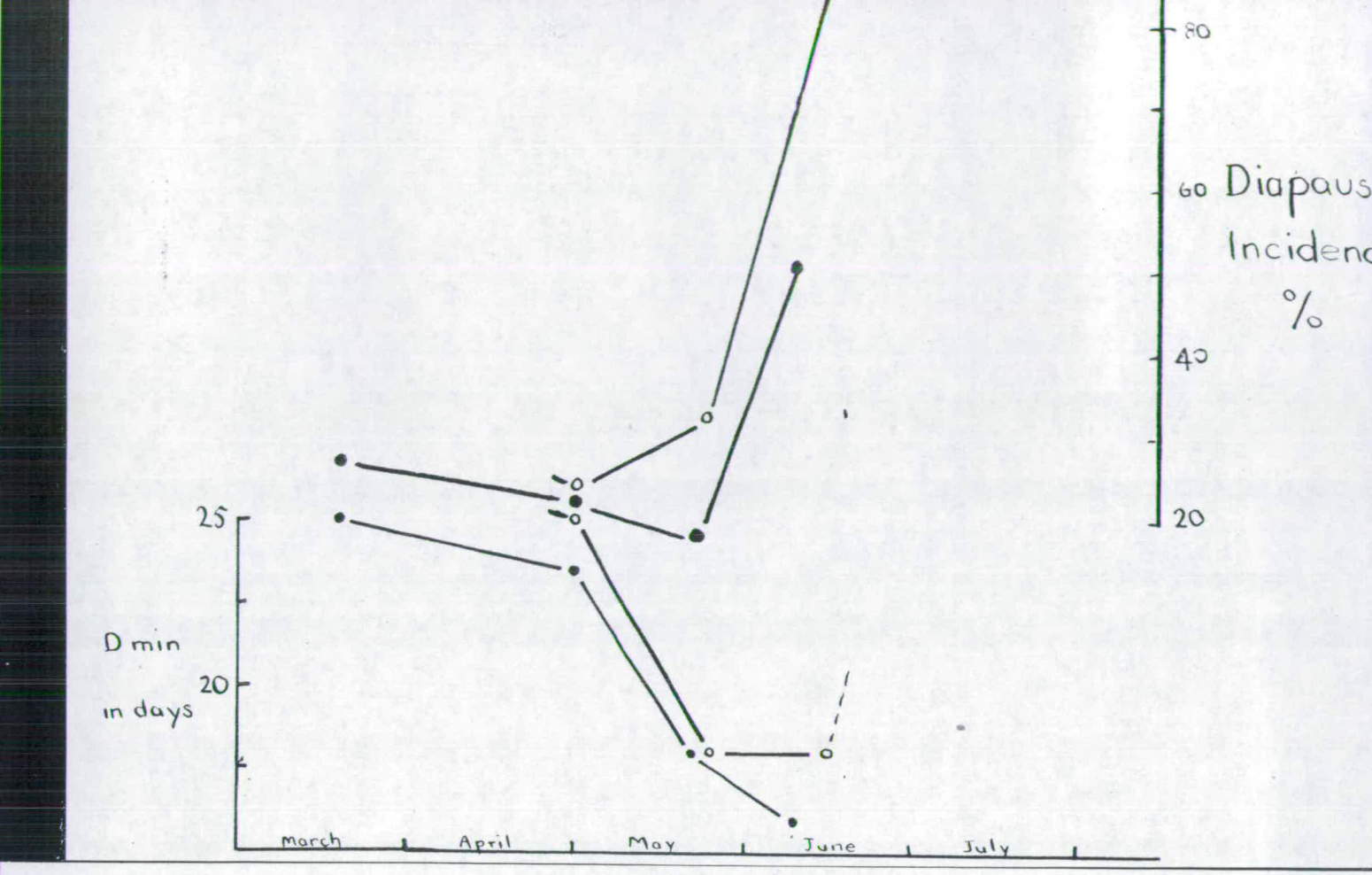
The seasonal rise and fall in diapause incidence was usually closely paralleled by an increase and decrease in the duration of the pre-moult. Dichotomy between these two occurs in the summer months, incidence rising while the minimum duration remains the same or is shorter. For example, diapause incidence in the Peebleshire nymphs rose between the 25th May and 6th June (18.7 to 51.3%) while the minimum duration fell from 18 to 17 days. The two criteria are compared for the larvae and nymphs in Figure 7. The dichotomy did not persist into July; the Perthshire nymphs delayed until the 19th of this month had a high incidence (99%) and a long pre-moult (minimum 69 days). The same phenomenon can be observed in the spring larvae although the increase in incidence occurred later. As absolute diapause became increasingly manifest, the duration of the pre-moult in the remainder was still short. Unlike the nymphs this was repeated in the early autumn populations incubated at 15°C. Diapause incidence was highest on the 2nd August (62%) and duration of the pre-moult shortest (minimum 22 days).

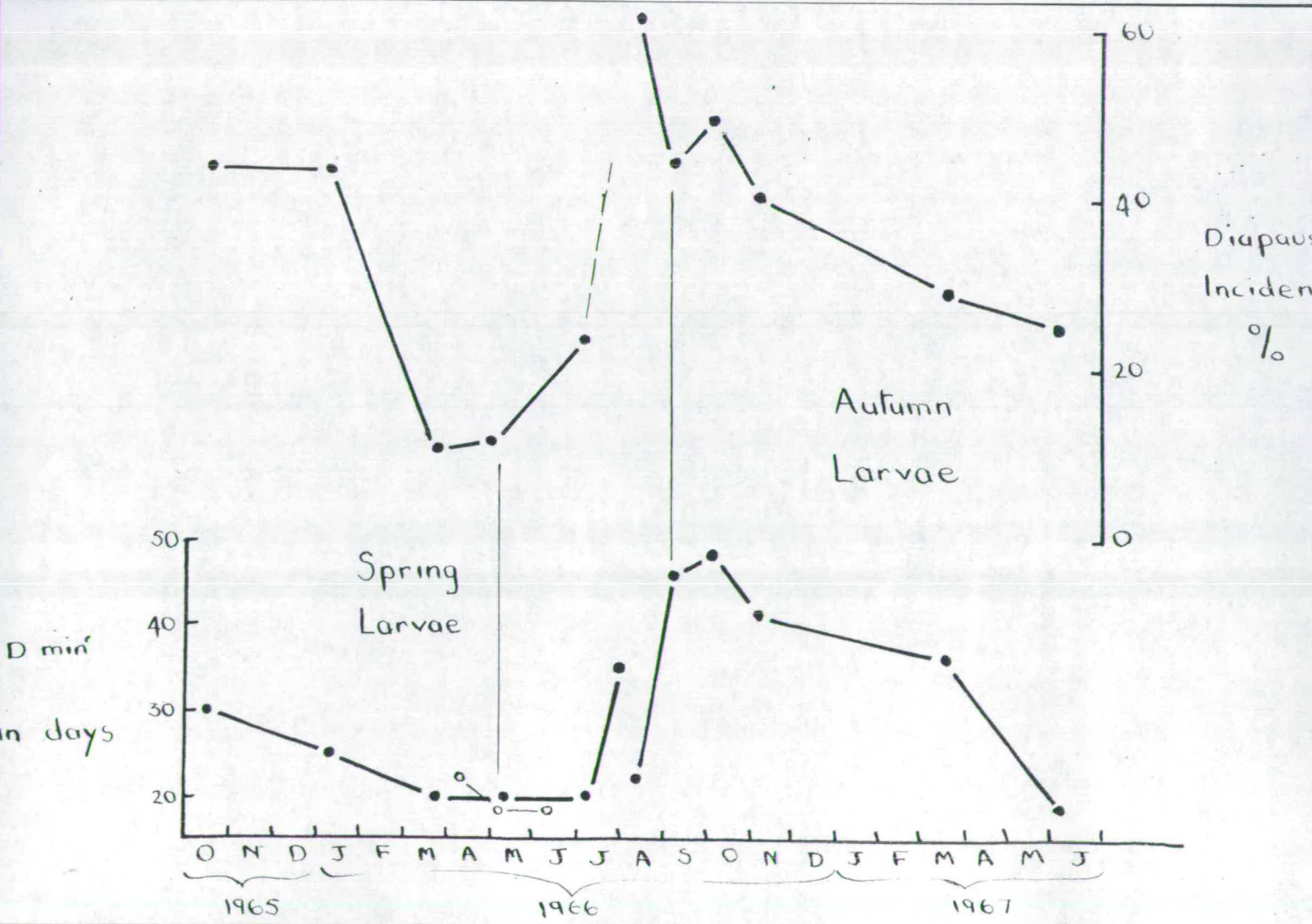
At a time of year when the conditions favourable for

/development

Figure 7. Top graph. The increase in diapause incidence and decrease in the minimum duration of the pre-moult in late May and June 1966. Open circles, Perthshire nymphs, closed circles, Peebleshire nymphs.

Bottom graph. The seasonal changes in the duration of the pre-moult compared withthe diapause incidence in tubed field larvae collected throughout the year. In midsummer the incidence of diapause begins to rise while development in the remaining ticks is completed rapidly. The open circles show the D. min. for larvae collected by dragging.


80
60
40
20
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Incidenc
%
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20
D min
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May
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July


60
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Larvae
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O N D J F M A M J J A S O N D J F M A M J
1965
1966
1967

development are becoming shorter there is an obvious advantage in an abrupt change from a very short developmental period to a prolonged diapause. The change occurs earlier for the ~~larvae~~ nymphs than the ~~nymphs~~ larvae. In similar observations on a Karelian population of I. ricinus Kheisin (1954) found rapid development (at 17 to 23°C.) in a proportion of the August larvae and diapause in the remainder.

A difference between the spring larvae confined in tubes and those collected at the vegetation tips was noted with regard to the minimum duration of the pre-moult. For the free larvae the minimum fell from 23 to 19 days from April to May; in the confined it remained from March to May at 20 days (Figure 7).

The combined data on diapause incidence and minimum duration of the pre-moult confirmed the view that development is characteristic of the seasonal experience of the hungry larvae irrespective of their season of origin. Thus spring larvae fed in autumn had a high diapause incidence and a prolonged pre-moult and autumn larvae the reverse when fed in spring (Figure 7).

3. The interaction of temperature and daylength on development of engorged larvae and nymphs.

Without using a multiplicity of temperature and light regimes it was possible to show that high temperatures and long days prevented absolute diapause and low temperatures and short days favoured it. A comparison between development of spring and autumn nymphs is given in Tables 4 and 5.

Table 4. The interaction of temperature and a long photoperiod (LD 18:6) on the development of spring and and autumn Perthshire nymphs.

DATE COLLECTED	NUMBER	TOTAL % IN MOULT PHASE AT; 10°C	15°C	18.5°C	23°C
Spring					
1st May, 1966	25-50	96	100	100	100
Autumn					
25th July 1965	22	-	5	95	100
27th Sept. "	25	-	50	100	100
17th Oct. "	21	0	45	100	100

Table 5. The interaction of temperature and short photoperiods (LD 14:10 to LD 10:14) on the development of spring and autumn Perthshire nymphs.

DATE COLLECTED	NUMBER	TOTAL % IN MOULT PHASE AT; 15°C LD 14:10	18.5°C LD 12:12	23°C LD 10:14
Spring				
1st May 1966	25-50	30	88	96
Autumn				
27th Sept. 1965	25	0	0	35
17th Oct. 1965	21	0	10	88

Few nymphs of a typical spring population (1st May 1966) were in diapause at 10°C and a long photoperiod (LD 18:6) whereas absolute diapause was broken in all autumn nymphs only at 18.5°C and above. The duration of the pre-moult was still prolonged at 18.5°C, up to 50 days for the September nymphs, but a maximum of only 26 days in May.

At short photoperiods a small proportion even of spring nymphs were in absolute diapause at high temperatures (Table 5). In autumn this proportion was much greater. The distinction between spring and autumn populations reported by Campbell (1948) is maintained here but the lower limiting temperature for development is also dependent on the photoperiodic conditions after engorgement.

/Short photoperiods

Short photoperiods not only maintain diapause, but tend to induce it directly, whether the incubation temperature or prior experience of the ticks favours diapause or not.(1st May population, Table 5). Conversely long days tend to break absolute diapause when other factors favour it (Table 4). In the silkworm Antherea pernyi Williams and Adkisson (1964) found that diapause could be maintained by the direct action of short days on chilled or unchilled pupae and averted by the direct action of long days. Apparently the diapausing stages of some arthropods respond to daylength in much the same way as the earlier 'sensitive stage'.

In the data for I. ricinus there was not a sufficient range of short photoperiods to determine the exact relationship between temperature and short days. In the autumn nymphs kept at a low temperature (15°C) diapause was manifest in the majority irrespective of the photoperiod. In spring, a temperature close to the threshold for development (10°C) did not completely eliminate the photoperiodic response (Table 6).

Table 6. The response to photoperiods in engorged larvae and nymphs of the Perthshire population incubated at low temperatures.

DATE COLLECTED	NUMBER OF TICKS	TOTAL % IN MOULT PHASE AT; 15°C		10°C	
		LD 18:6	LD 14:10	LD 18:6	LD 14:10
21st May 1967	20 nys.	100	80	90	65
26th June 1967	48 lar.	100	50	92	13

Observations on spring ticks at 10°C gave some additional information on the seasonal characteristics of development. The minimum duration of the pre-moult was shortest in Peebleshire nymphs collected in June. Absolute diapause at a long photoperiod (LD 18:6) appeared as early as April and by the 26th May

/when diapause

when diapause incidence was lowest at 15°C, 19% of the nymphs were already unable to develop at 10°C (Table 7). The lower limiting temperature for development begins to rise very early in the season.

Table 7. Development of Peebleshire nymphs incubated at 10°C and a long photoperiod (LD 18:6).

DATE COLLECTED IN 1966	NUMBER	TOTAL % IN MOULT PHASE AT 10°C AFTER; 40 days	50 days	75 days	100 days	125 days
16th-23rd Mar.	32	0	0	90	100	100
30th April	50	0	0	98	98	98
26th May	50	0	4	81	81	81
6th June	20	0	20	20	20	20

The earlier appearance of diapause at 10°C was also found for the larvae; only 3.1% of those fed on the 3rd July entered the moult phase while at 15°C diapause incidence was still low (24%) (Table 8).

Table 8. Development of tubed field larvae incubated at 10°C and a long photoperiod (LD 18:6).

DATE COLLECTED IN 1966	NUMBER	TOTAL % IN MOULT PHASE AFTER 150 DAYS AT 10°C LD 18:6	MINIMUM DURATION OF THE PRE-MOULT IN DAYS
23rd March	52	100	45-55
11th May	133	96	45-55
3rd July	790	3.1	95

The seasonal temperature relations of nymphal development at a long photoperiod are summarised in Figure 8. The graph merely indicates the seasonal change and includes data from collections made in Peebleshire in spring 1966 and Perthshire in autumn 1965.

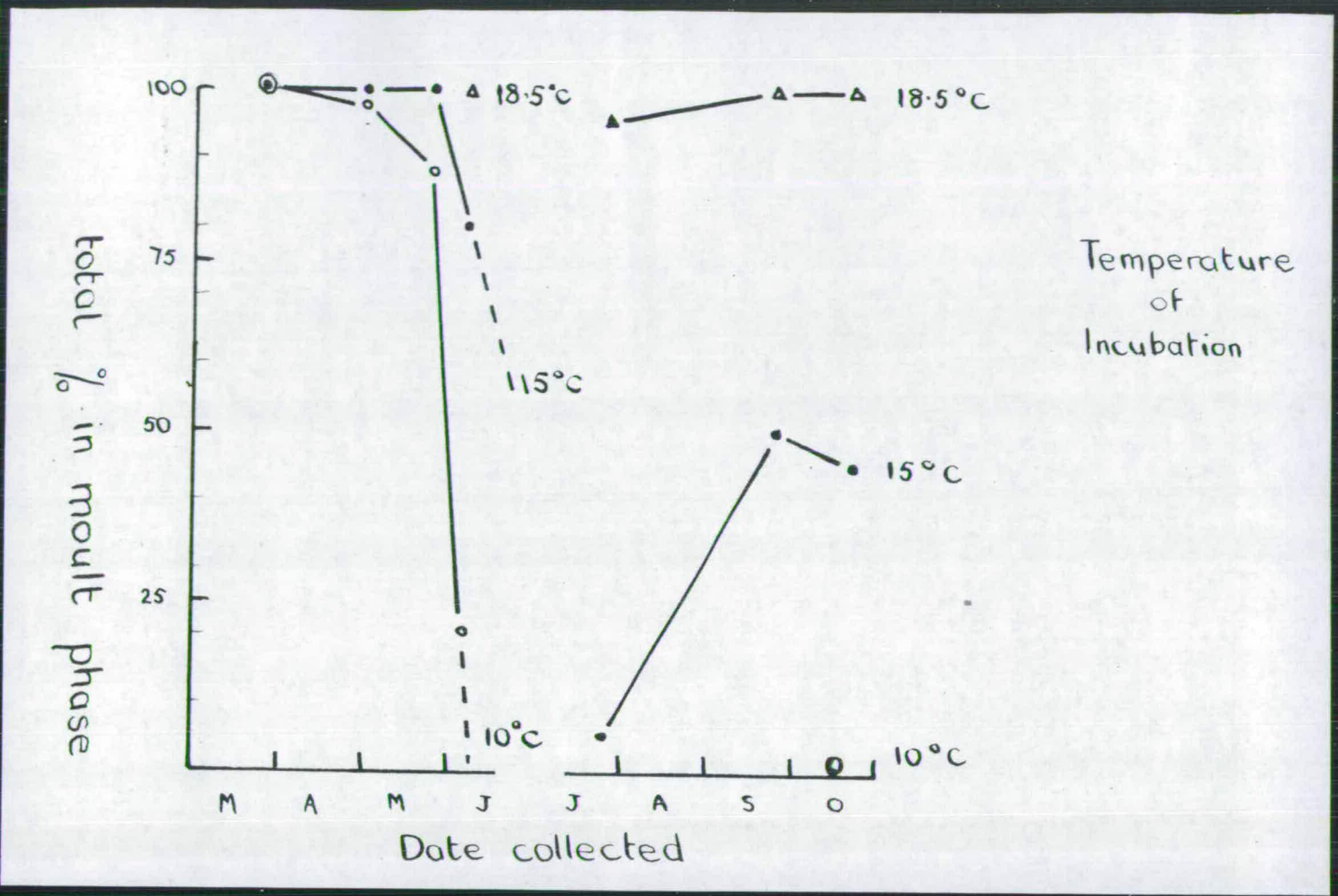


Figure 8. The temperature relations of spring and autumn nymphs kept at an 18 hour photoperiod after feeding. Spring ticks were collected in Peebleshire 1966, autumn ticks in Perthshire, 1965.

4. Summary of the observations on the seasonal rhythm of development in larvae and nymphs.

The absence of a more complete range of temperatures and photoperiods has tended to obscure the temperature and photoperiodic relations of the engorged ticks.

It is possible that with the rise in lower limiting temperature during spring and summer there is a rise in the temperature at which the threshold photoperiod is clearly defined. Towards the lower limiting temperature diapause would appear at long days, and at high temperatures diapause would be prevented at short days; both would tend to obscure the threshold. For example the majority of nymphs collected in October 1965 were in diapause at 15°C; at 18.5°C there was a threshold period between 18 and 12 hours of light per day, but at 23°C the threshold was again obscure, the majority entering the moult phase at a short photoperiod (Tables 4 and 5). Determination of the threshold value at a constant temperature (15°C) would show an increase in diapause incidence and threshold photoperiod as the lower limiting temperature rose to approach this temperature (15°C), in the spring and summer months. Conversely incidence and threshold (at 15°C) would fall from autumn to spring as the lower limiting temperature falls from 15°C to below 10°C.

It is true that within many populations a shift in the threshold can be expected at higher incubation temperatures. For example, of the nymphs collected in Perthshire on the 28th May, 1966, only 46% entered the moult phase at 10°C and an 18 hour photoperiod, whereas the threshold photoperiod was below 16 hours of light per day at 15°C. The total percentages in moult phase at 18, 16 and 14 hours of light per day were 100,

/76 and 28 per cent

76, and 28 per cent respectively. Nevertheless the results were sufficiently comprehensive to show that a rising temperature requirement for development and for determination of a sharply defined threshold photoperiod were not adequate explanations for the seasonal changes observed. The lower limiting temperature at a photoperiod of 18 hours per day was clearly rising for the Peebleshire nymphs collected in spring 1966 (Figure 8). While the threshold photoperiod was well defined between 16 and 14 hours of light per day at 15°C in the early nymphs (March-April), it became obscured on the 26th May, not by the appearance of diapause but the reverse, an absence of diapause at short photoperiods (Figure 4). By the 6th June the threshold was ill-defined, but individuals in absolute diapause were found at all photoperiods. There was no indication of a sharp threshold at 10°, 15° or 18.5°C. The total in moult phase at 10°C and 18 hours of light per day was 20%; at 15°C and 18, 16 and 14 hours of light per day it was 80%, 53% and 12% respectivley; at 18.5°C and 18 and 12 hours of light per day it was 100% and 65% respectively.

The criteria which have been used to describe development at 15°C, diapause incidence, threshold photoperiod and minimum duration of the pre-moult all achieved their highest levels some time in autumn. The lower limiting temperature for development was higher for larvae in July and for nymphs from July to September. These diapause phenomena reached their lowest levels in spring and rise again in early summer. The lower limiting temperature was already higher for some of the nymphs

/fed in

fed in April and the larvae fed in May. The diapause incidence rose sharply in June for the nymphs and in July for the larvae. The duration of the pre-moult in June and July was as short or shorter than earlier fed ticks; only in late July was the pre-moult prolonged.

5. Seasonal changes in the duration of the pre-oviposition and embryonic periods at different temperatures and daylengths.

The duration of the pre-oviposition period at 10°, 15° and 18.5°C was not influenced by the length of the photoperiod. A delay associated with the summer and autumn experience of hungry adults was consistently found at an incubation temperature of 15°C. Females collected in Perthshire on the 13th May 1966 began laying after 13 to 18 days. When collected on the 28th June oviposition began after 22 to 28 days and on the 22nd July after 18-42 days. In each collection from 22 to 28 females were observed. These results are entirely in agreement with Campbell's extensive data for collections made in 1945 (Campbell 1948).

The influence of daylength on the duration of embryonic development was somewhat enigmatic. Weekly batches of eggs were laid at 15°C by females collected in Peebleshire on the 6th April 1965. The first larvae emerged after 68 to 88 days (Figure 9). In all eggs incubated at a long photoperiod (LD 18:6) or in darkness the first larvae were seen between these days, but at short photoperiods (LD 14:10 and LD 6:18) larvae were not found in many batches until 160 to 170 days after laying. Even at 18.5°C a short photoperiod again delayed

/development

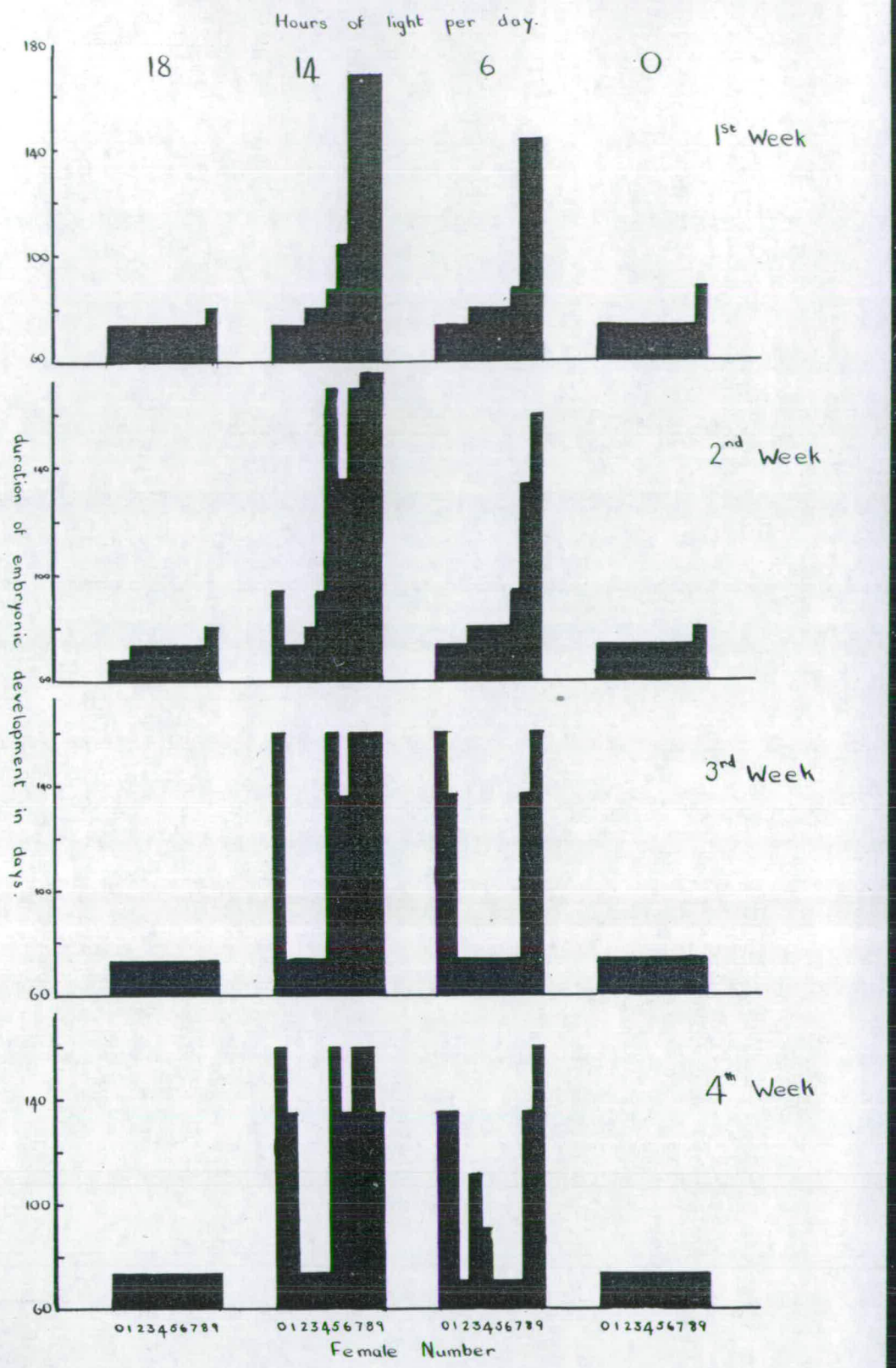


Figure 9. The influence of daylength on the duration of embryonic development at 15°C. Both the engorged females and their eggs were incubated at the same photoperiod.

Egg batches were separated from the females every week and the parent number is given at the bottom of the histograms.

Engorged females were collected in Peebleshire 6-4-65.

development (Figure 10). At both temperatures the whole period from engorgement of the females to the emergence of the larvae was under the same light conditions. The photoperiod may have been recognised by the female or by the embryo. In experiments with later collections, eggs which were laid at a short photoperiod (LD 14:10) were moved immediately to a long photoperiod (LD 18:6). Although a delay was found in some batches at short days, the response was too inconsistent to distinguish between these and the eggs transferred to long days. Development of spring eggs at short day conditions is shown in Figures 11, 12 and 13.

Recent evidence has shown that diapause occurred during early embryonic development. Eggs were delayed in a stage of early blastoderm formation (Kemp 1967) approximating to Wagner's stages 10-12 in the embryonic development of Ixodes calcaratus Birula, 1894 (= Boophilus annulatus (Say, 1821)), Wagner (1894). It seems unlikely that at this early stage the embryo could respond to daylength conditions and a more plausible explanation would be for the control of spring diapause to reside within the maternal generation.

The midsummer experience of the unfed adult certainly influences development of their eggs. Larvae began to emerge after 61 to 68 days at 15°C in the majority of egg batches laid by females collected in Perthshire on the 13th May 1966. Females which were delayed in feeding until the 28th June laid eggs with a prolonged diapause, larvae emerging after 122 to 182 days (Figure 13). These females were undoubtedly delayed spring

/ticks

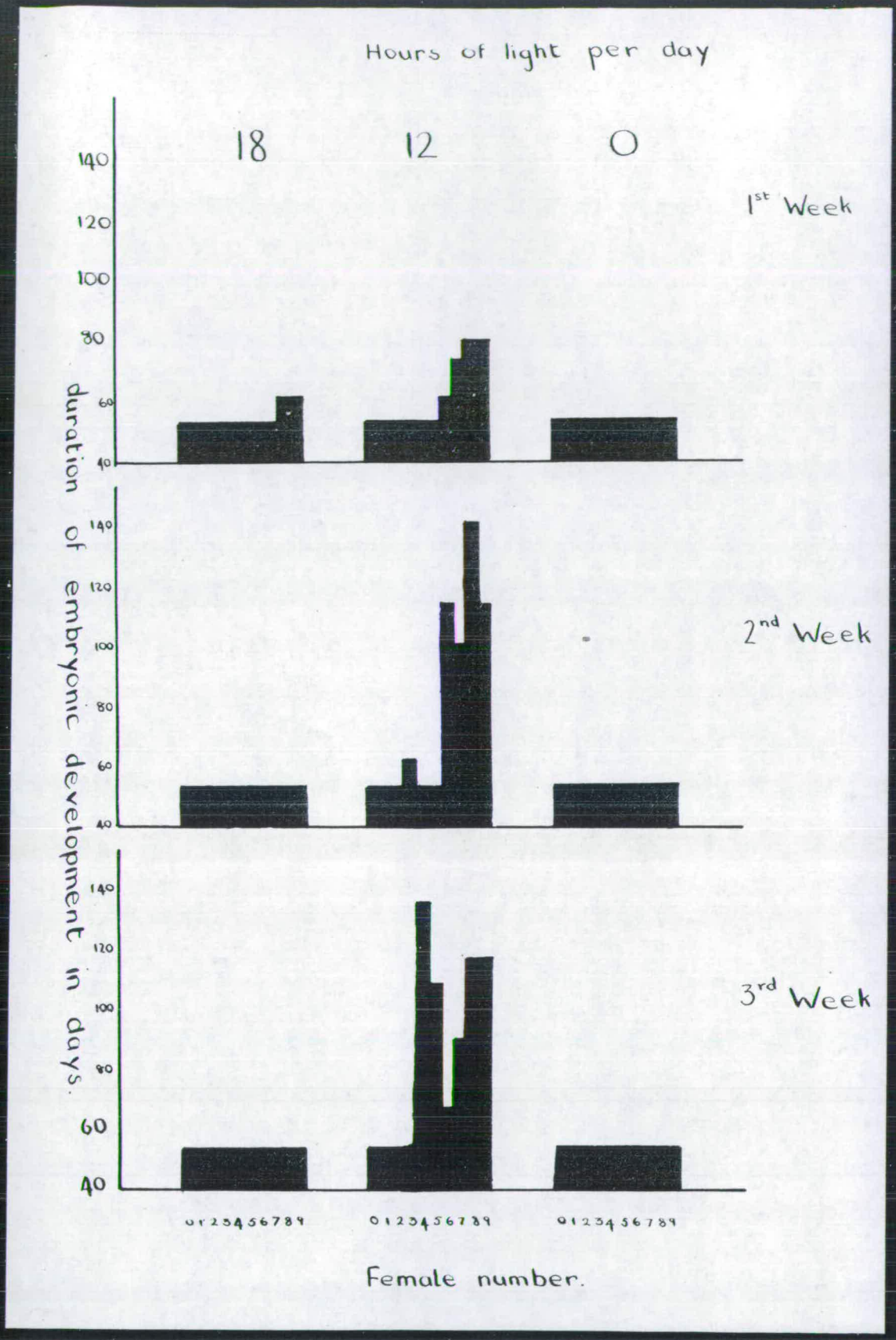


Figure 10. The influence of daylength on the duration of embryonic development at 18.5°C. Both the engorged females and their eggs were incubated at the same photoperiod. Egg batches were separated from the females each week, and the parent number is given at the bottom of the histograms.

Engorged females were collected in Peebleshire 6-4-65.

ticks; the first autumn ticks to emerge in the field in 1966 and 1967 were larvae at the end of June 1967 and nymphs and adults in July 1966. Campbell (1948) did not find autumn adults ready to feed until the end of July.

Diapause occurred in the majority of egg batches laid by females collected in autumn 1965 and 1966 (Figures 12 and 13). The size of the samples was too small and diapause too variable to permit an analysis of the pre-feeding seasonal or post-feeding photoperiodic influence on development.

A consistent and prolonged embryonic diapause is associated with the autumn compared with the early spring experience of the unfed adults. Unlike the larvae and nymphs there was no tendency for a long photoperiod (LD 18:6) to break diapause in autumn eggs incubated at 15°C.

In direct contrast with the larvae and nymphs autumn diapause was virtually absent in eggs which were laid and incubated at 10°C. Larvae began to emerge after 235 to 261 days (mean 244.7 days) in 16 egg batches incubated at 10°C and a long photoperiod, LD 18:6. At a short photoperiod LD 14:10 emergence from 16 batches began between 235 and 268 days (mean 246 days). All 32 females were collected on the 22nd July 1966. In 30 egg batches from spring females incubated at 10°C and a long photoperiod emergence of the first larvae was found between 197 and 256 days (mean 228.8 days).

This result is consistent with Campbell's data for 1945, when the prolongation of autumn embryonic development never exceeded 12% of the incubation period for spring eggs laid and incubated at 10°C.

/Section IV

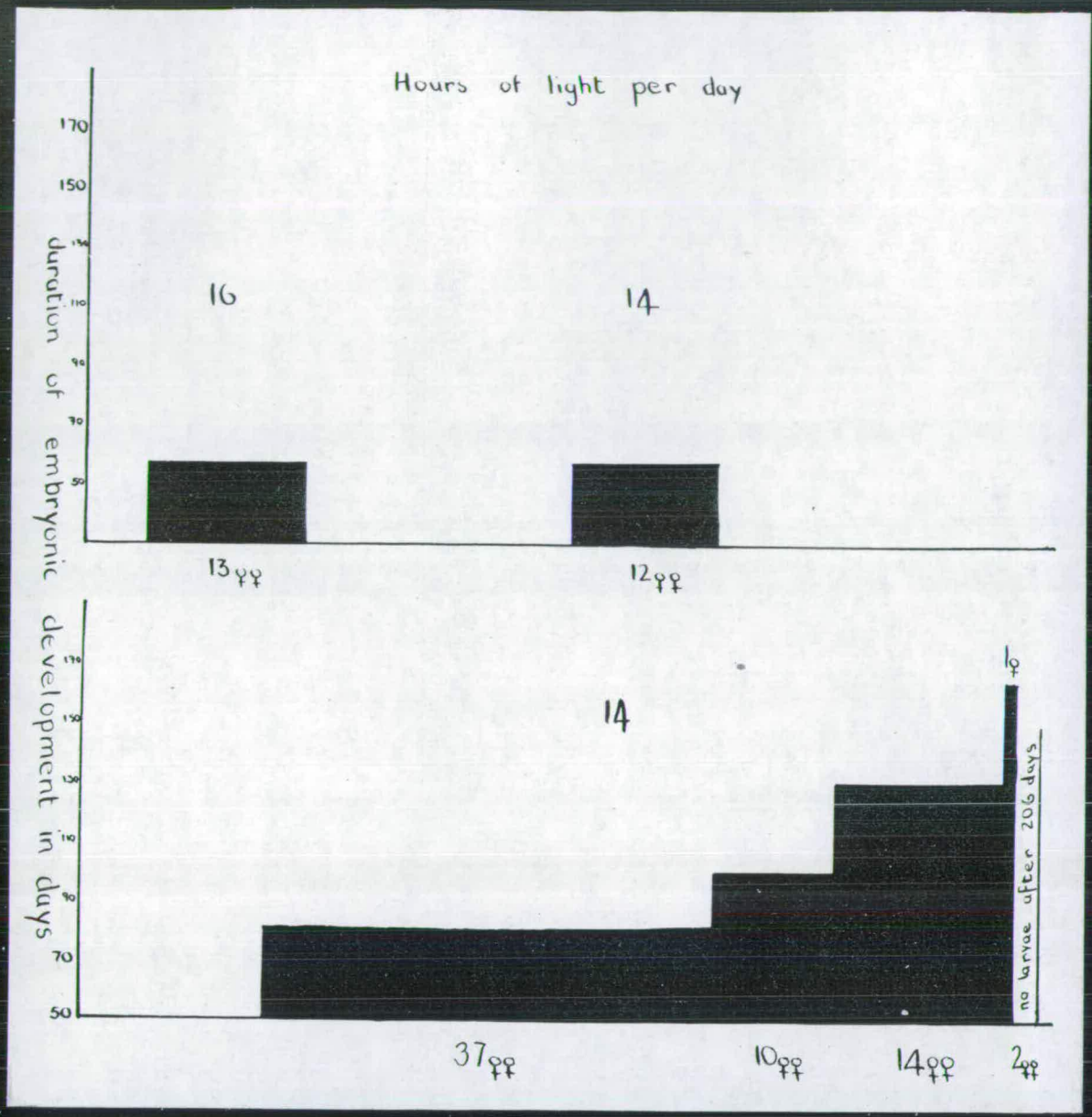


Figure 11. The influence of daylength on the duration of embryonic development at 15°C. The eggs in the top graph were derived from females collected in Perthshire in April 1967 and in the bottom graph from females collected in Argyllshire in April 1965.

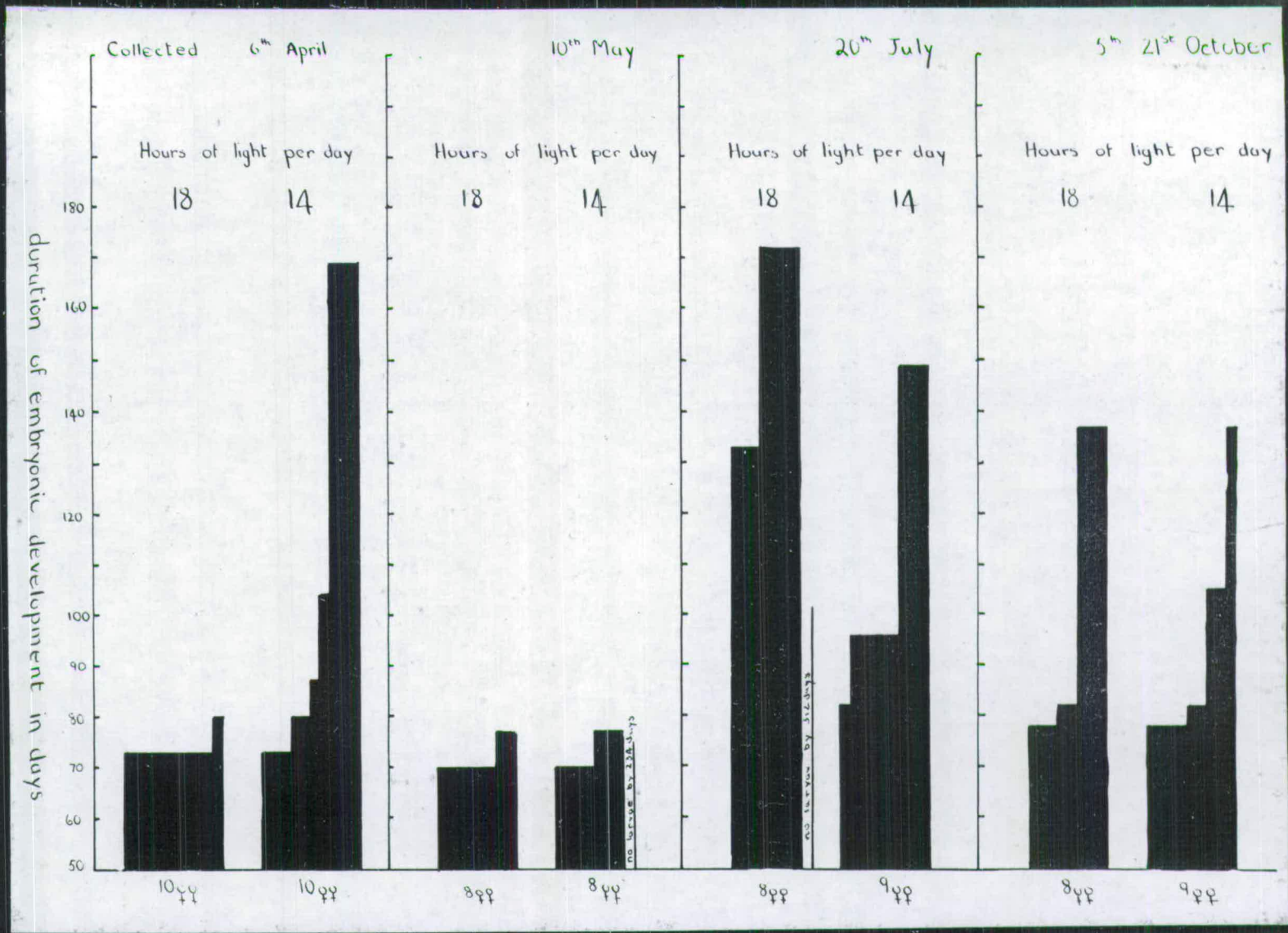


Figure 12. A comparison of spring and autumn embryonic development at 15°C and a long (LD 18:6) and a short (LD 14:10) photoperiod. The dates at the top indicate when the engorged females were collected. The 6th April adults were from Peebleshire, the rest from Perthshire.

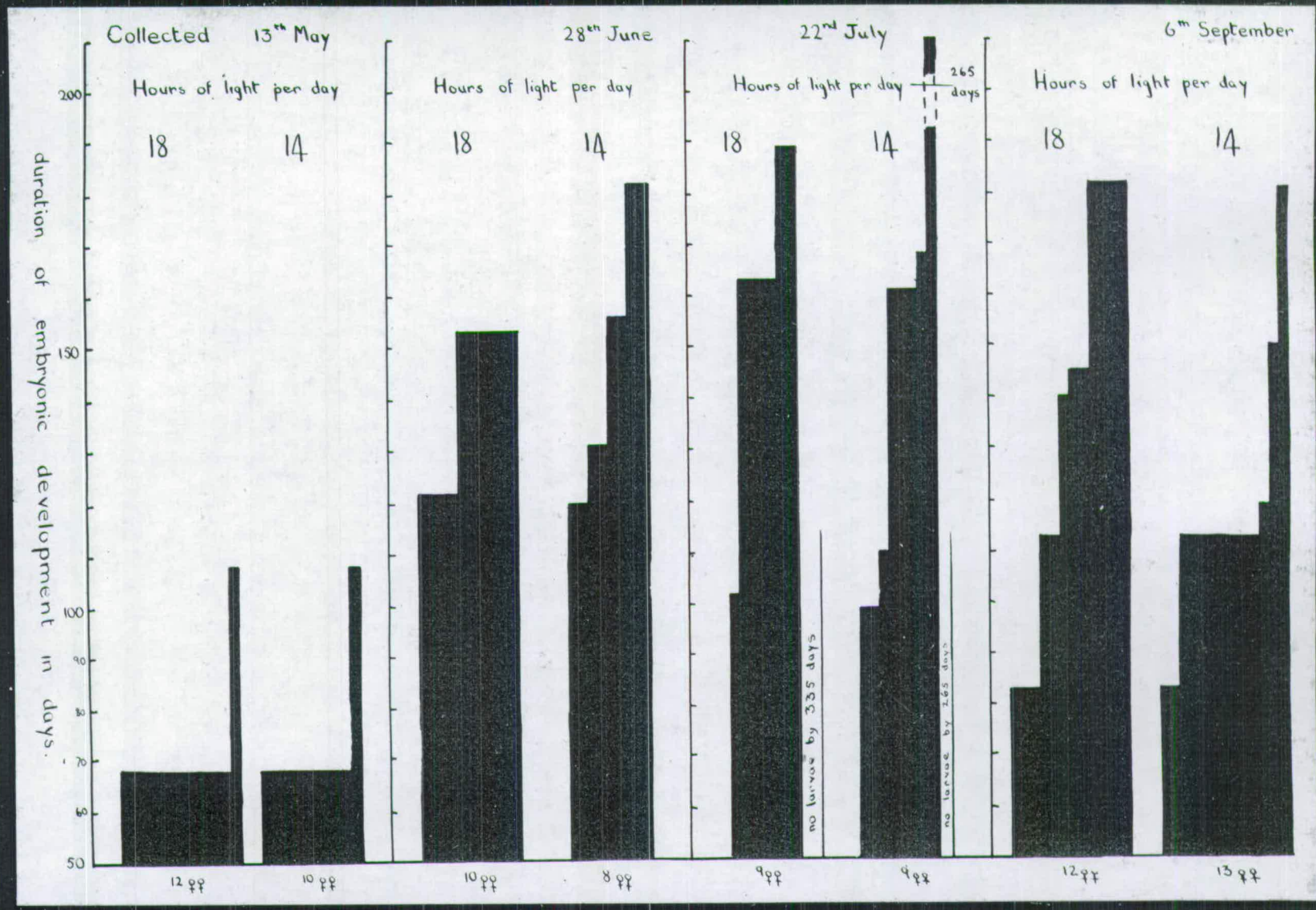


Figure 13. A comparison of spring and autumn embryonic development at 15°C and a long (LD 18:6) and a short (LD 14:10) photoperiod. 1966.

IV

THE DEPENDENCE OF DEVELOPMENT IN LABORATORY REARED TICKS ON THE TEMPERATURE AND PHOTOPERIODIC CONDITIONS EXPERIENCED BEFORE AND AFTER FEEDING

1. The response to long and short days experienced by unfed larvae and nymphs.

Spring females were incubated at 15°C and an 18 hour photoperiod. Their larvae were fed 4 to 40 weeks after emergence and again incubated at 15°C. The 4 week old larvae had a lower incidence of diapause than the 12 week or older larvae (Figure 14). More striking was the very short pre-moult in the youngest group, a minimum of 18 days, comparable with the spring field ticks with a minimum of 19 to 20 days. There was in all age groups a high diapause incidence, 67% to 83.3%; spring field larvae ranged from 12% to 24%.

Diapause was most strongly expressed after 12 weeks experience of long days (83.3%) and fell gradually to 68.7% after 40 weeks exposure. Few larvae were fed successfully earlier than 4 weeks and few survived longer than 40 weeks.

Substantially the same results were obtained when these experiments were repeated using larvae from autumn females or larvae reared for one generation at 15°C in the laboratory (Figures 15 and 16). There was a greater distinction between the younger and older larvae in these populations, but the incidence of diapause was still far higher than in the minimum found in the field ticks (44.7% compared with 11%).

There was a tendency for diapause induction to break down after one generation in the laboratory. The last graph in Figure 16 shows the relative absence of diapause in 12 week old

/larvae

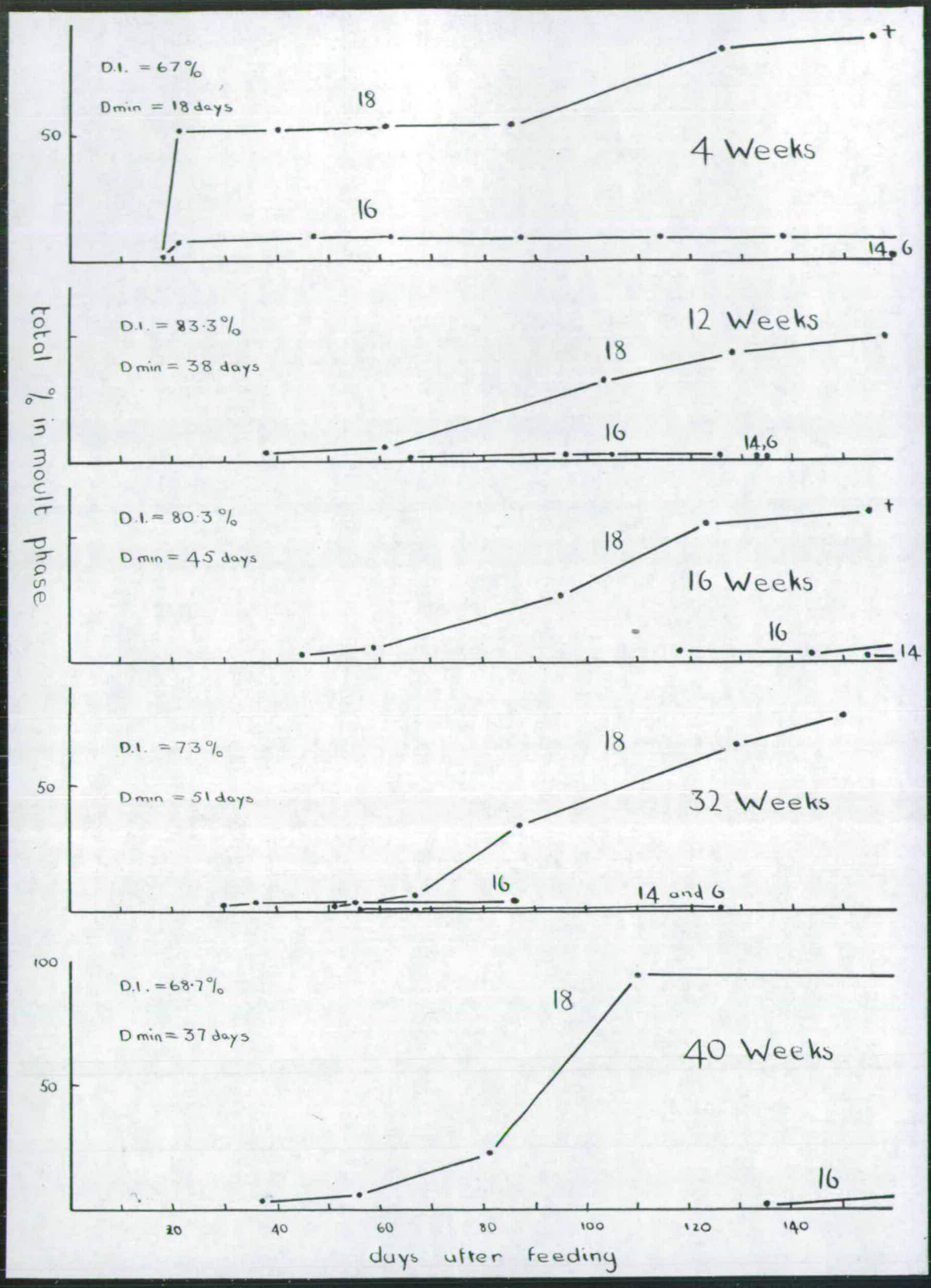


Figure 14. Development at 15°C of larvae derived from spring collected females. The larvae experienced from 4 to 40 weeks at 15°C LD 18:6 before feeding.

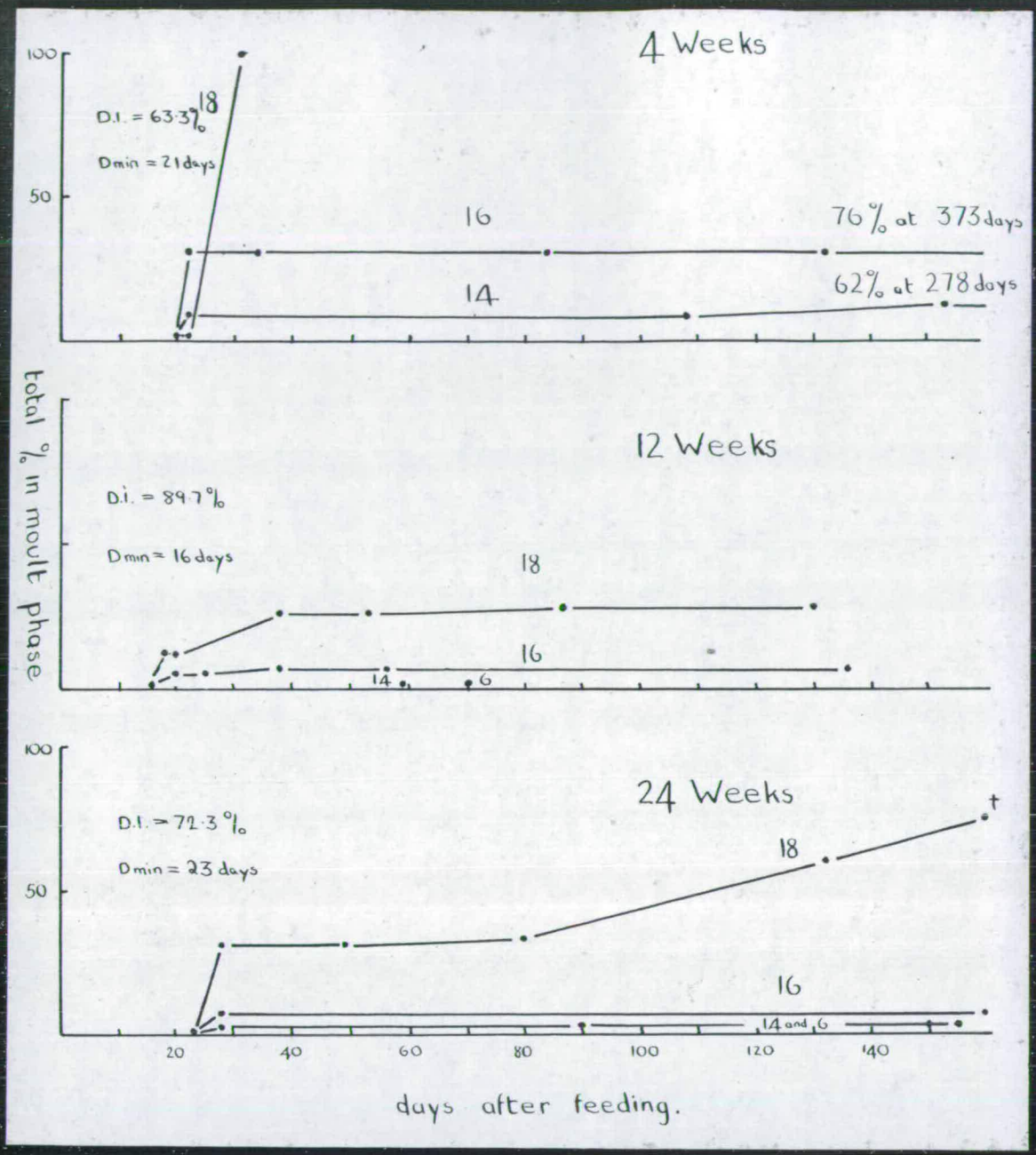


Figure 15. Development at 15°C of larvae derived from autumn collected females. The larvae experienced from 4 to 24 weeks at 15°C LD 18:6 before feeding.

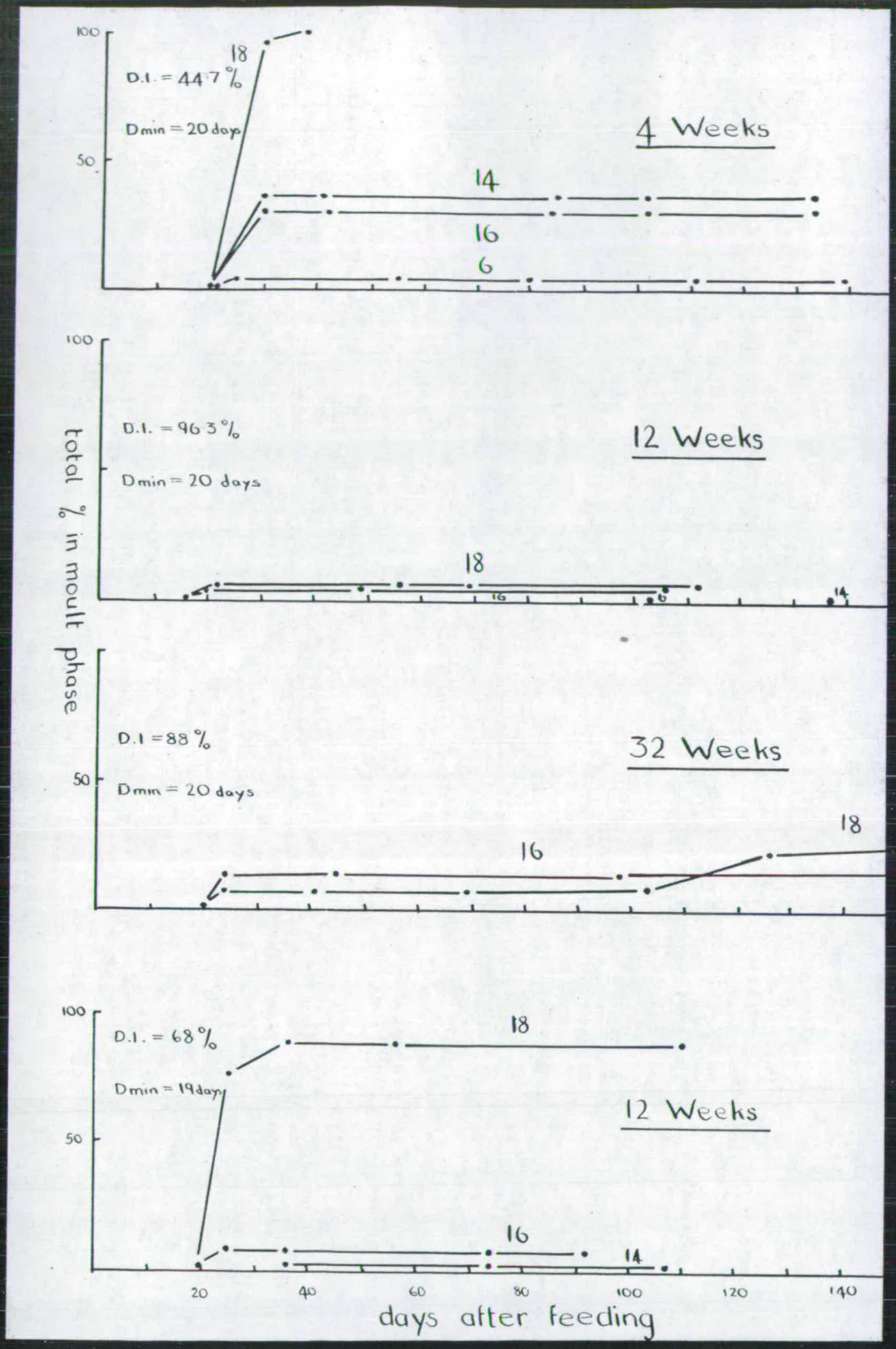


Figure 16. Development at 15°C of larvae derived from ticks reared in the laboratory for one generation (reared at 15°C). The larvae experienced from 4 to 32 weeks at 15°C LD 18:6. The last graphs show the lower diapause incidence in some of these populations.

larvae of a different first generation population.

Spontaneous recovery from diapause was quite common in some of these larvae. The first graph in Figure 15 gives the percentages which eventually entered the moult phase.

Conditioning the larvae at a short photoperiod (LD 6:18) had quite a different effect on development. In the spring derived larvae there was a progressive decline in diapause incidence and threshold photoperiod with increasing exposure (Figure 17). The minimum duration of the pre-moult was invariably prolonged (30-44 days). However diapause incidence fell to comparable levels with the spring field populations (i.e. 6.7% compared with 11%), and the threshold fell below 14 hours of light per day.

A repeat of this experiment using larvae from autumn females gave a less satisfactory result (Figure 18). The larvae fed after 4 weeks experience already had a relatively low incidence of diapause, and this did not decline progressively with increasing experience of short days.

A short photoperiod of 14 hours per day was also effective in lowering diapause incidence (Figure 19).

An improvement was found in the larvae derived from spring females with an increase in the conditioning period from 6 to 20 weeks but thereafter incidence and duration fluctuated although the threshold photoperiod was always below 16 hours per day. Larvae from autumn females showed a similar improvement with an increase from 4 to 20 weeks exposure (Figure 20).

/The diapause

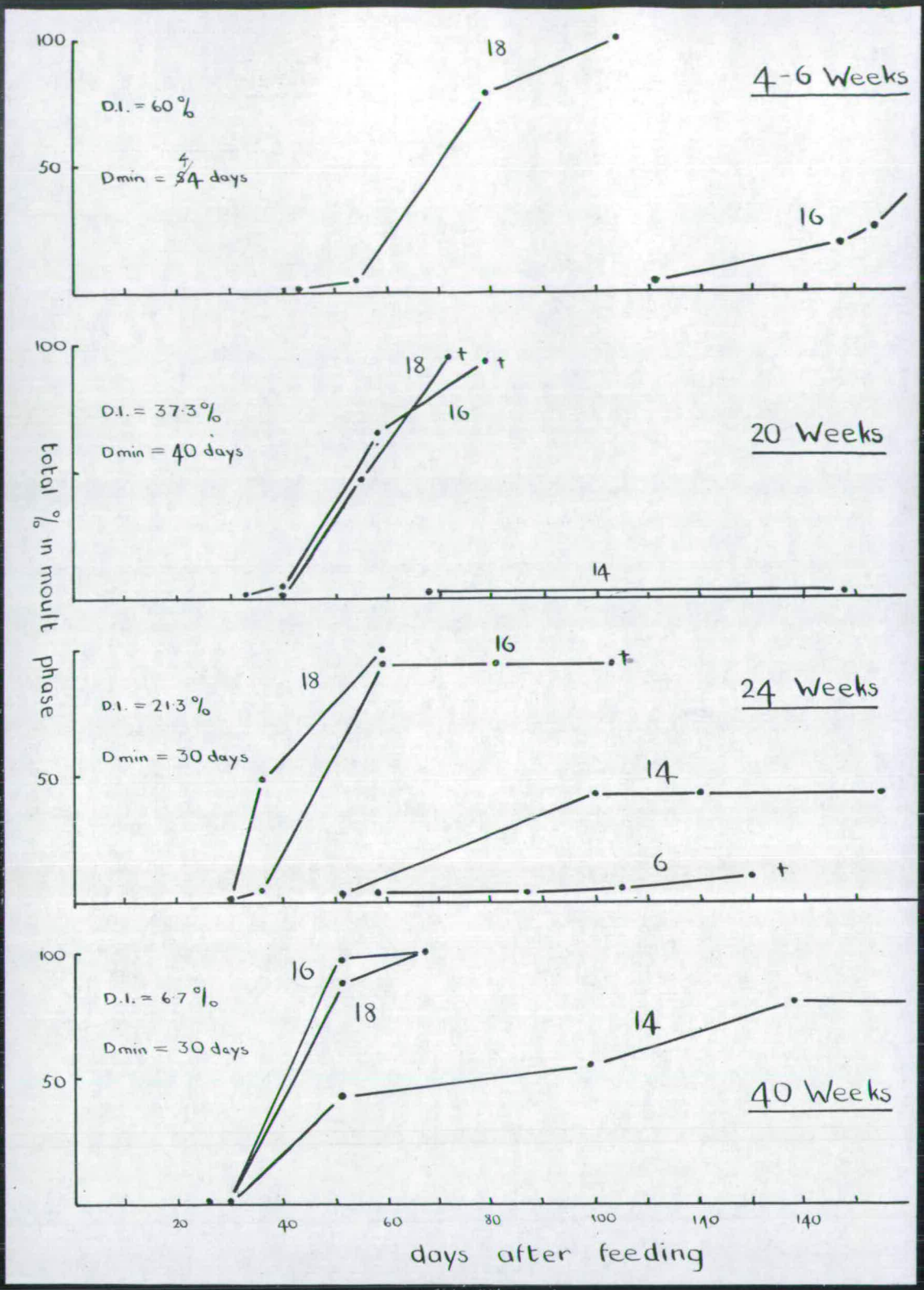


Figure 17. Development at 15°C of larvae derived from spring females The larvae experienced from 4 to 40 weeks at 15°C LD 6:18 before feeding.

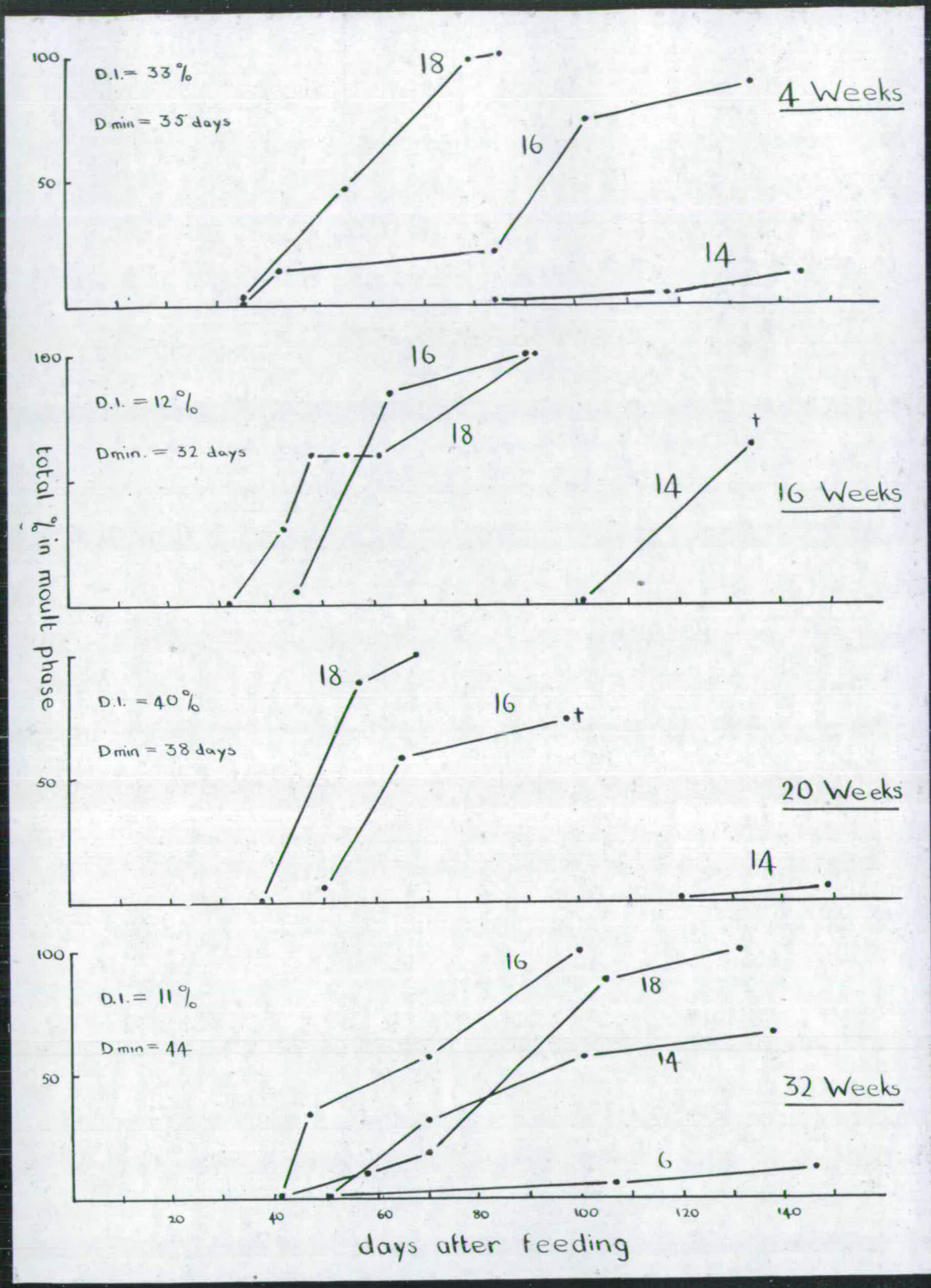


Figure 18. Development at 15°C of larvae derived from autumn females
The larvae experienced from 4 to 32 weeks at 15°C LD 6:18 before feed

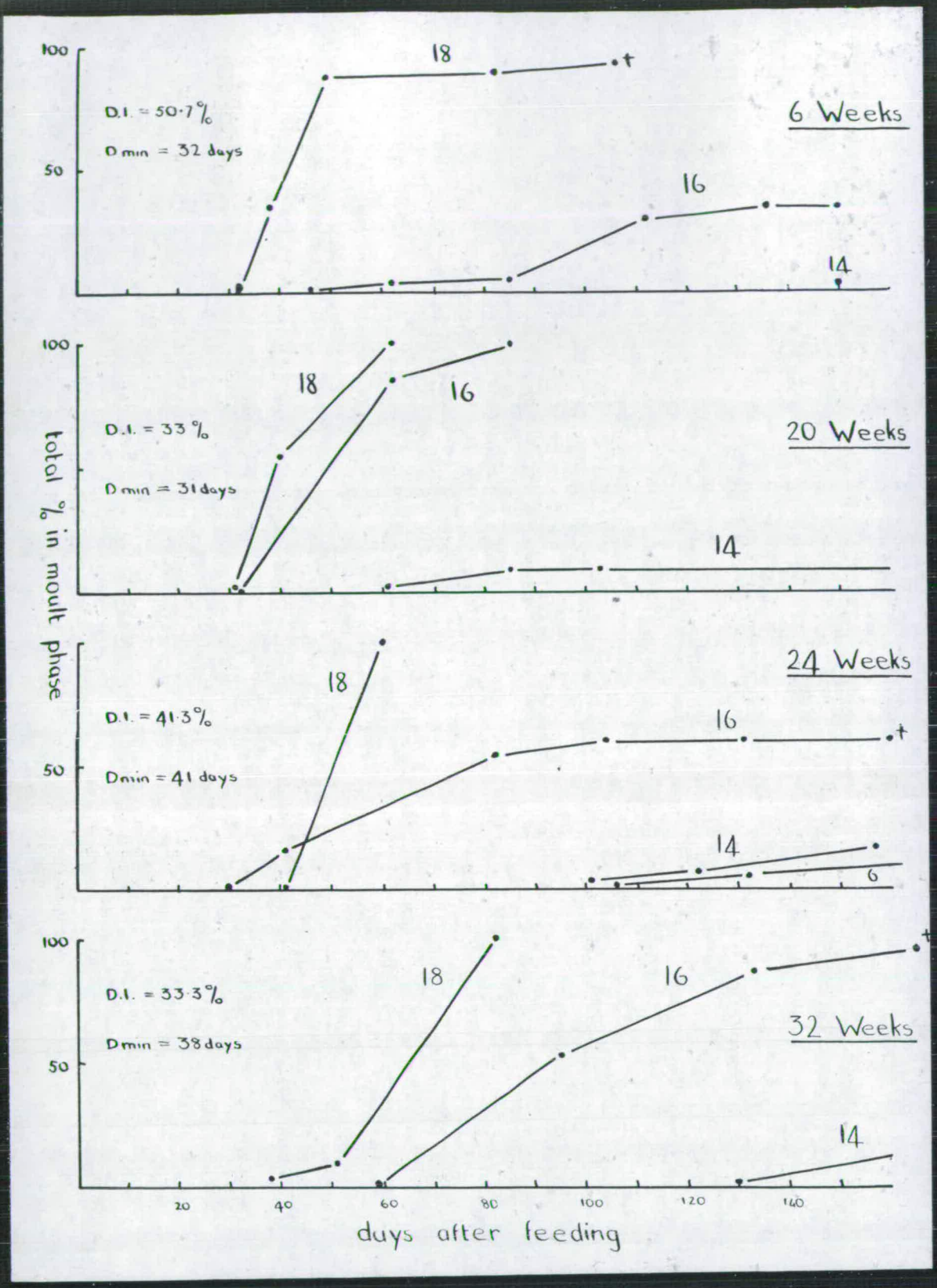


Figure 19. Development at 15°C of larvae derived from spring females. The larvae experienced from 6 to 32 weeks at 15°C and LD 14:10 before feeding.

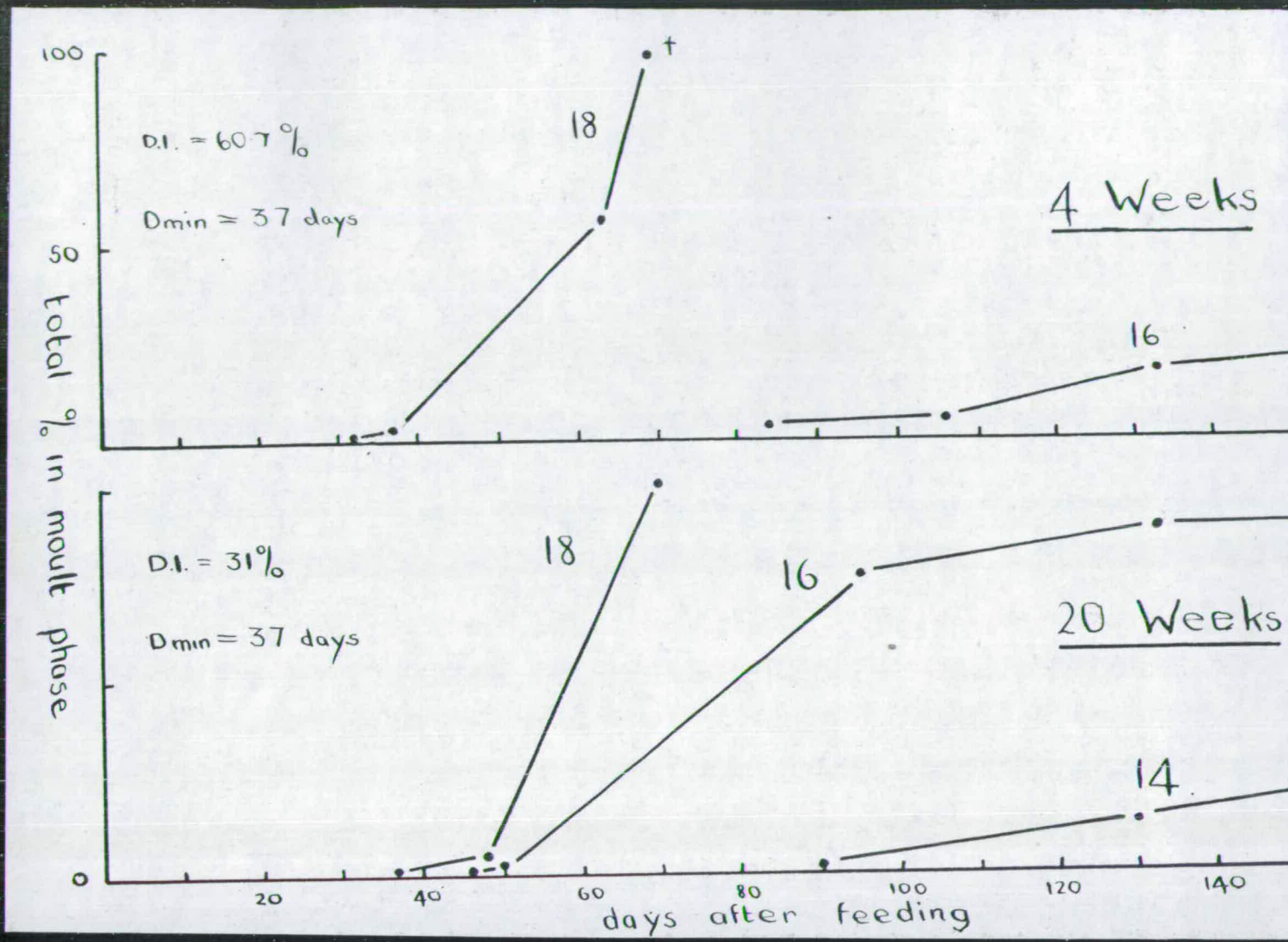


Figure 20. Development at 15°C of larvae derived from autumn females. The larvae experienced 4 or 20 weeks at 15°C LD 14:10 before feeding.

The diapause incidence and threshold photoperiods for all the larval experiments are summarised in Figure 21. The youngest long and short day larvae differed only in the minimum duration of the pre-moult. Between the first and second long-day samples within each population there was invariably an increase in diapause incidence, and in the short day samples invariably a decrease. There was at least two hours difference in the threshold photoperiod for the older larvae.

It must be emphasised that these differences obtain only at a post-feeding temperature of 15°C. At a higher incubation temperature few larvae were in diapause after brief exposure to long days. Development at 18.5°C is shown in Table 9 for the same long day larvae whose development at 15°C is recorded in Figure 14. Diapause was virtually absent in these younger larvae, and the strong tendency for diapause to be broken largely obscured the increasing tendency to diapause found at a lower incubation temperature. Experience of short days still prolonged the pre-moult phase.

Table 9. Development of engorged larvae derived from spring females. The larvae were conditioned at 15°C before feeding and incubated at 18.5°C.

PHOTOPERIODIC EXPERIENCE AT 15°C BEFORE FEEDING	TOTAL % IN MOULT PHASE AFTER 100 DAYS AT 18.5°C		MINIMUM DURATION OF THE PRE-MOULT IN DAYS
	LD 18:6	LD 12:12	
LD 18:6 for 4 weeks	100	86	12
LD 18:6 for 24 weeks	100	21	13
LD 14:10 for 4 weks	100	18	21

(This experiment was terminated after 100 days, when most of the diapausing individuals had died).

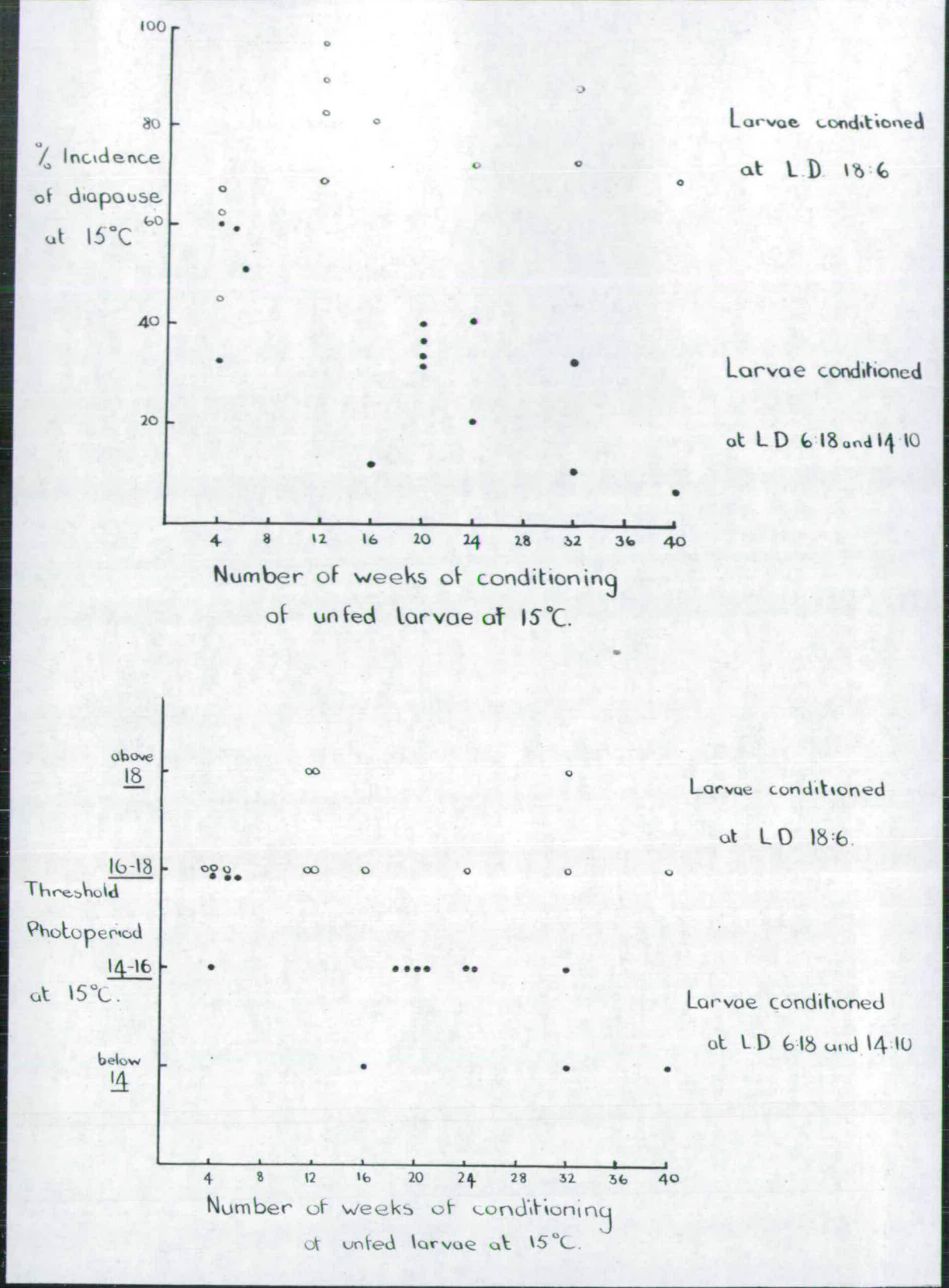


Figure 21. Top graph. The incidence of diapause at 15°C in larvae conditioned before feeding at long (LD 18:6) or short (LD 14:10 or LD 6:18) photoperiods.
Bottom graph. The post-feeding threshold photoperiod at 15°C, of these same larval populations.

In laboratory reared ticks, only larvae conditioned for many weeks at short days showed appreciable development after 200 days incubation at 10°C and an 18 hour photoperiod (Table 10). Recovery from diapause was frequently found after about 250 days irrespective of prior conditioning.

Table 10. Development of engorged larvae derived from autumn females. The larvae were conditioned at 15°C before feeding and incubated at 10°C.

PHOTOPERIODIC EXPERIENCE AT 15°C BEFORE FEEDING	TOTAL % IN MOULT PHASE AFTER 200 DAYS AT 10°C	MINIMUM DURATION OF THE PRE-MOULT IN DAYS
	LD 18:6	
4 weeks at LD 18:6	0	230-240
24 " " " " "	0	240-266
4 weeks at LD 14:10	0	220-230
24 " " " " "	69	118
4 weeks at LD 6:18	0	209-216
24 " " " " "	82	105

The results at an incubation temperature of 10°C supported the conclusion that prolonged short day conditioning lowers diapause incidence and also the lower limiting temperature for development.

The threshold in the response of unfed larvae to photoperiod lies between 16 and 14 hours of light per day. After 8 weeks experience of a 16 hour photoperiod, diapause incidence was high (65.3%) and the minimum duration of the pre-moult very short, 19 days (Figure 22). Development was characteristic for larvae exposed to long days. Prolonged experience of total darkness was similar in effect to short days, the pre-moult was delayed (minimum 28 days) and diapause incidence low (36.7%)

/(Figure 22)

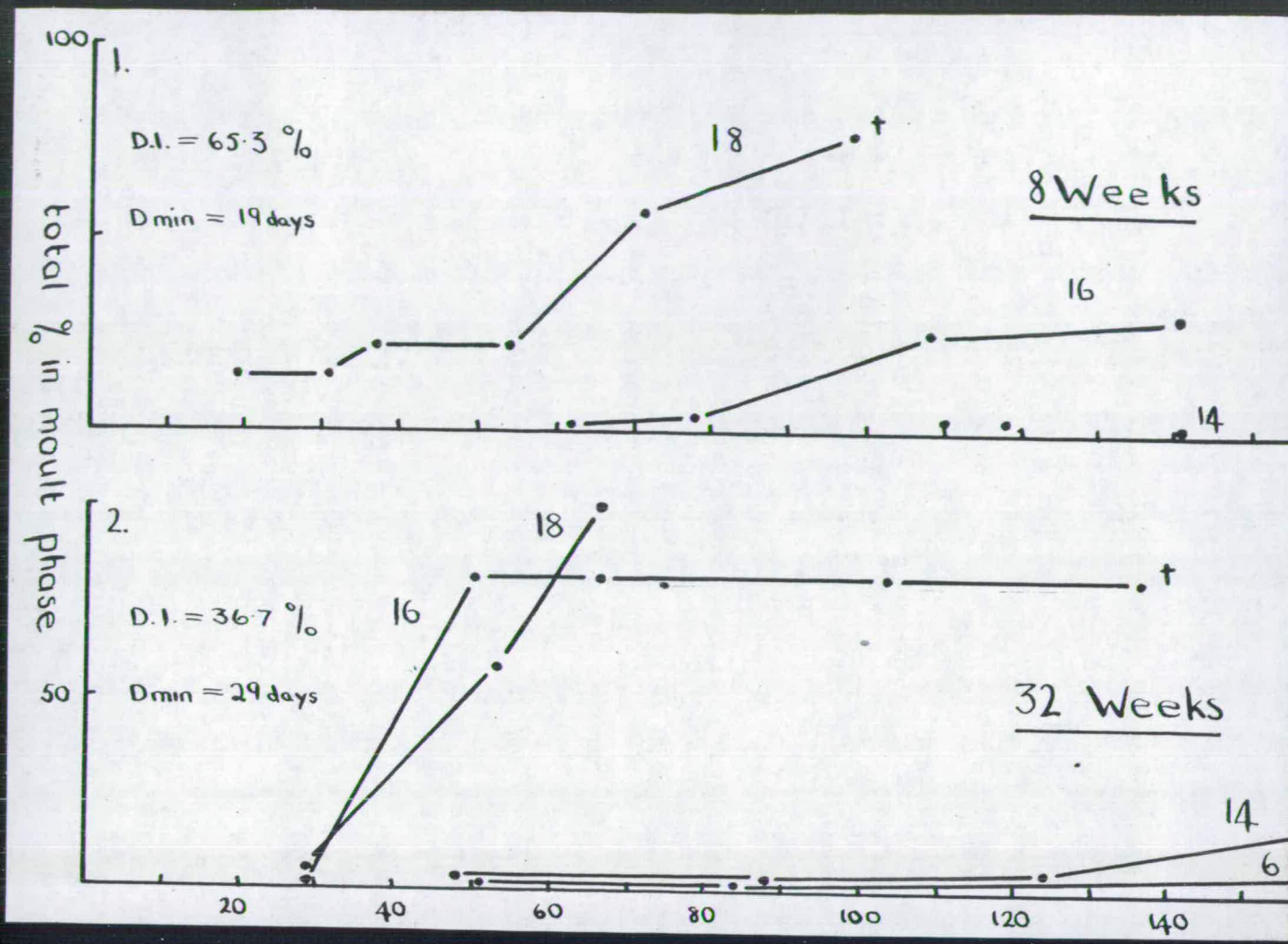


Figure 22. Graph 1. Development at 15°C of larvae conditioned before feeding for 8 weeks at 15°C LD 16:8.
Graph 2. Development at 15°C of larvae conditioned before feeding for 32 weeks at 15°C LD 0:24.

(Figure 22).

Laboratory reared nymphs had a higher temperature threshold than the larvae. Nymphs which emerged from spring larvae at long days were conditioned for 4 to 24 weeks before feeding. Diapause was strongly manifest in all ticks incubated at 15°C; only at an 18 hour photoperiod did a number of nymphs enter the moult phase. The minimum duration of the pre-moult was 118 days (Table 11).

Table 11. Development of engorged nymphs at 15°C and an 18 Hour photoperiod. Nymphs were derived from spring Larvae and experienced from 4 to 24 weeks at a long photoperiod before feeding.

DURATION OF EXPOSURE TO LD 18:6 AT 15°C BEFORE FEEDING	NUMBER	TOTAL % IN MOULT PHASE AFTER 200 DAYS AT 15°C AND LD 18:6	MINIMUM DURATION OF THE PRE-MOULT IN DAYS
4 weeks	28	65	132
6 "	25	0	-
8 "	20	20	169
24 "	31	16	137

Nymphs which were derived from autumn larvae experienced 6 and 16 weeks of long days and were incubated at 15°C and 18.5°C after feeding. Again few ticks developed at 15°C but in both groups absolute diapause was broken at 18.5°C(Table 12).

Table 12. Development of engorged nymphs at 15°C and 18.5°C . Nymphs were derived from autumn larvae and experienced 6 and 16 weeks at a long photoperiod before feeding.

DURATION OF EXPOSURE TO LD 18:6 AT 15°C BEFORE FEEDING	NUMBER	TOTAL % IN MOULT PHASE AFTER 100 DAYS AT;			MINIMUM DURATION OF THE PRE-MOULT AT 18.5°C AND LD 18:6
		15°C LD 18:6	18.5°C LD 18:6	18.5°C LD 12:12	
6 weeks	31	0	97	9	25 days
16 "	24	0	96	0	25 "

/(The experiment ----

(The experiment was terminated after 100 days; many nymphs died at the higher temperature after this time.)

An increasing tendency to diapause after prolonged experience of long days was not revealed at an incubation temperature of 15°C (Table 11) and there were too few experiments at the higher temperature to draw any conclusions on the similarity between larvae and nymphs on this point. At 18.5°C absolute diapause was broken in both young and old nymphs; the post-feeding photoperiod again influenced development.

Nymphs which fed 4 to 6 weeks after emergence at short day conditions also had a high incidence of diapause (Table 13).

Table 13. Development of engorged nymphs at 15°C and a long photoperiod (LD 18:6). The nymphs were derived from autumn and spring larvae and experienced short days before feeding.

ORIGIN	PHOTOPERIODIC CONDITIONS AT 15°C BEFORE FEEDING	NUMBER	TOTAL % IN MOULT PHASE AFTER 200 DAYS AT 15°C LD 18:6	MINIMUM DURATION OF THE PRE-MOULT
Autumn larvae	4-6 weeks at LD 6:18	34	15	93 days
Spring larvae	4-6 weeks at LD 14:10	28	53	96 "

Prolonged exposure of these same populations lowered the incidence of diapause at 15°C almost to spring field levels; 27.7% after 24 weeks at LD 6:8 and 33.3% after 24 weeks at LD 14:10 (Figure 23). Total darkness was less effective than short days (diapause incidence 56%), but was nearer to the short than the long day response (Figure 23).

The threshold between the long and short day response was again between 16 and 14 hours of light per day. After 24

/weeks

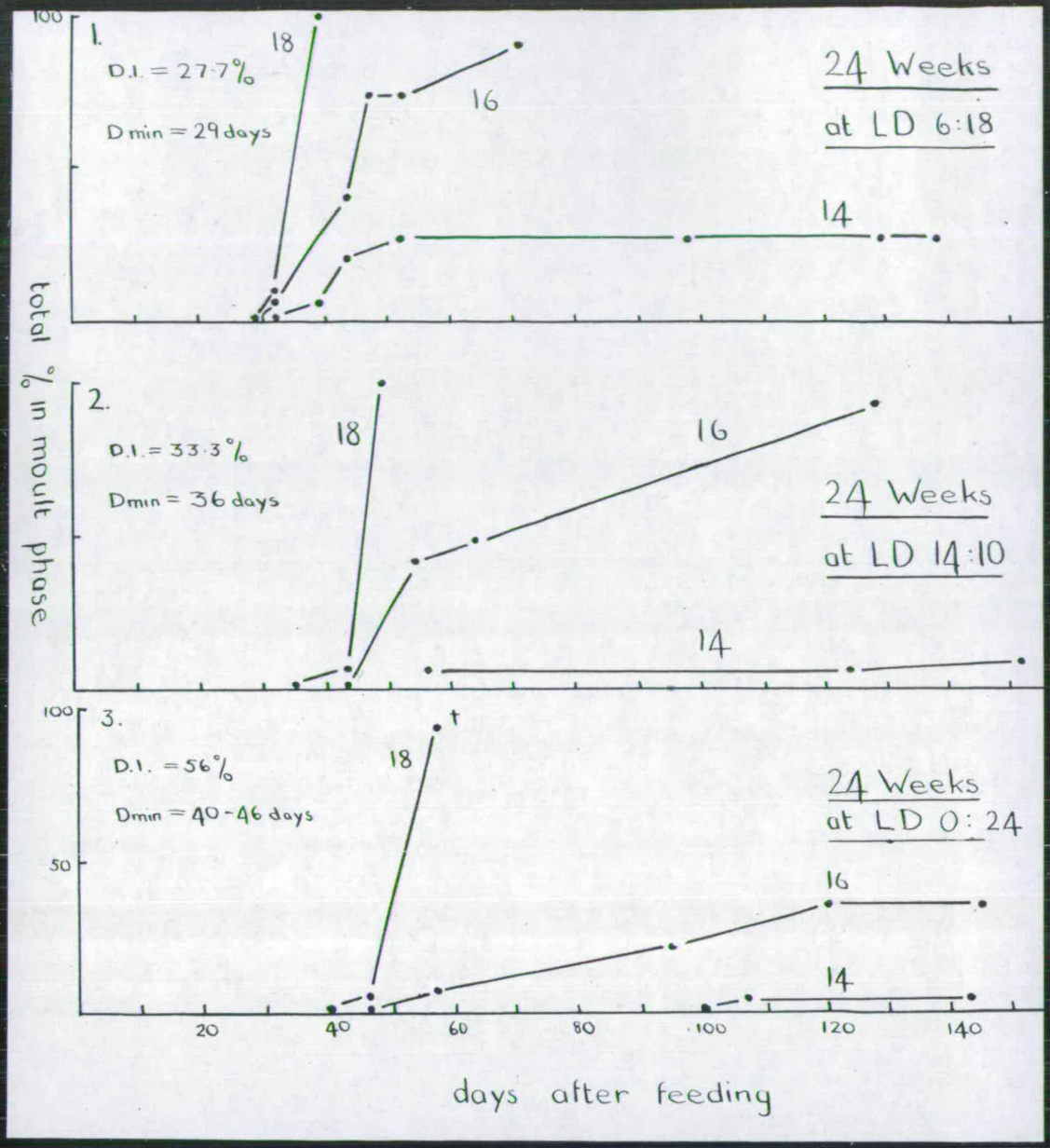


Figure 23. Development of nymphs at 15°C.
Graph 1. Nymphs conditioned at 15°C LD 6:18 for 24 weeks before feeding.
Graph 2. Nymphs conditioned at 15°C LD 14:10 " " " " "
Graph 3. Nymphs conditioned at 15°C LD 0:24 " " " " "

weeks at a 16 hour photoperiod diapause incidence was 90% and the minimum duration of the pre-moult 61 days.

2. The interaction of temperature and photoperiod in diapause induction.

When pre-oviposition, oviposition and embryonic development were allowed to proceed slowly at 10°C the first larvae began to emerge 200 days or more after laying. They were unwilling to attach but after 6-8 weeks a small part of the population fed successfully. Farther batches derived from the same spring population of females were fed after 12 and 24 weeks conditioning. There was virtually no difference after experience of a long (LD 18:6) or a short (LD 14:10) photoperiod (Figure 24). The early 6-8 week larvae conditioned at LD 18:6 did have a higher incidence of diapause than their short day fellows (80% compared with 48.3%). But after 24 weeks exposure these fell to 30% and 25.7% respectively. The influence of long day conditioning at 10°C was very different from its effect at 15°C; diapause incidence was low and the pre-moult never less than 25 days. Presumably photoperiods were not recognised or the low temperature had a stronger counter effect.

Larvae which experienced a long photoperiod at 23°C and 18.5°C again had a high incidence of diapause at 15°C (Figures 25 and 26). The larvae were derived from the same population which were conditioned at 15°C (Figure 14). The results were

/substantially

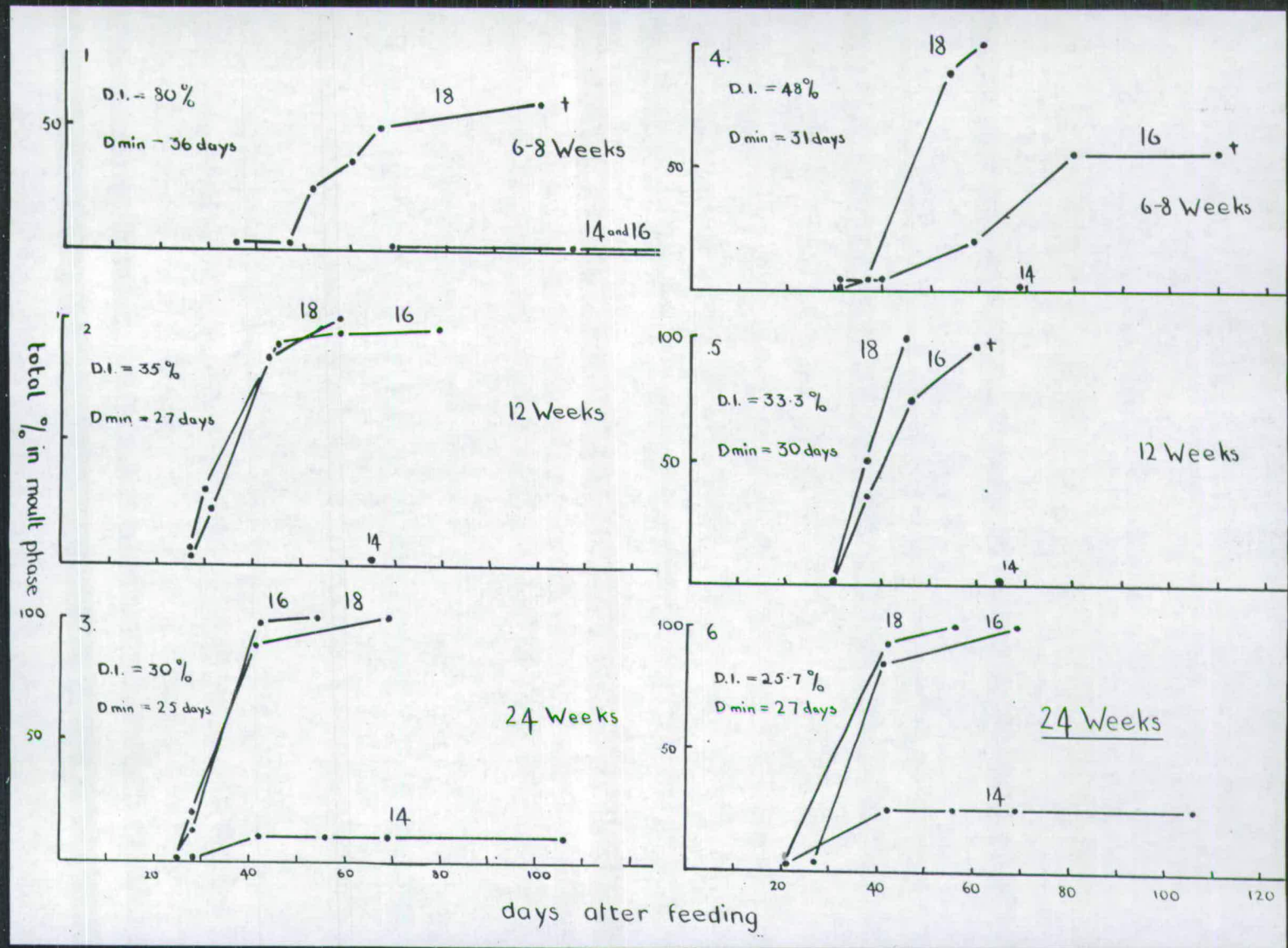
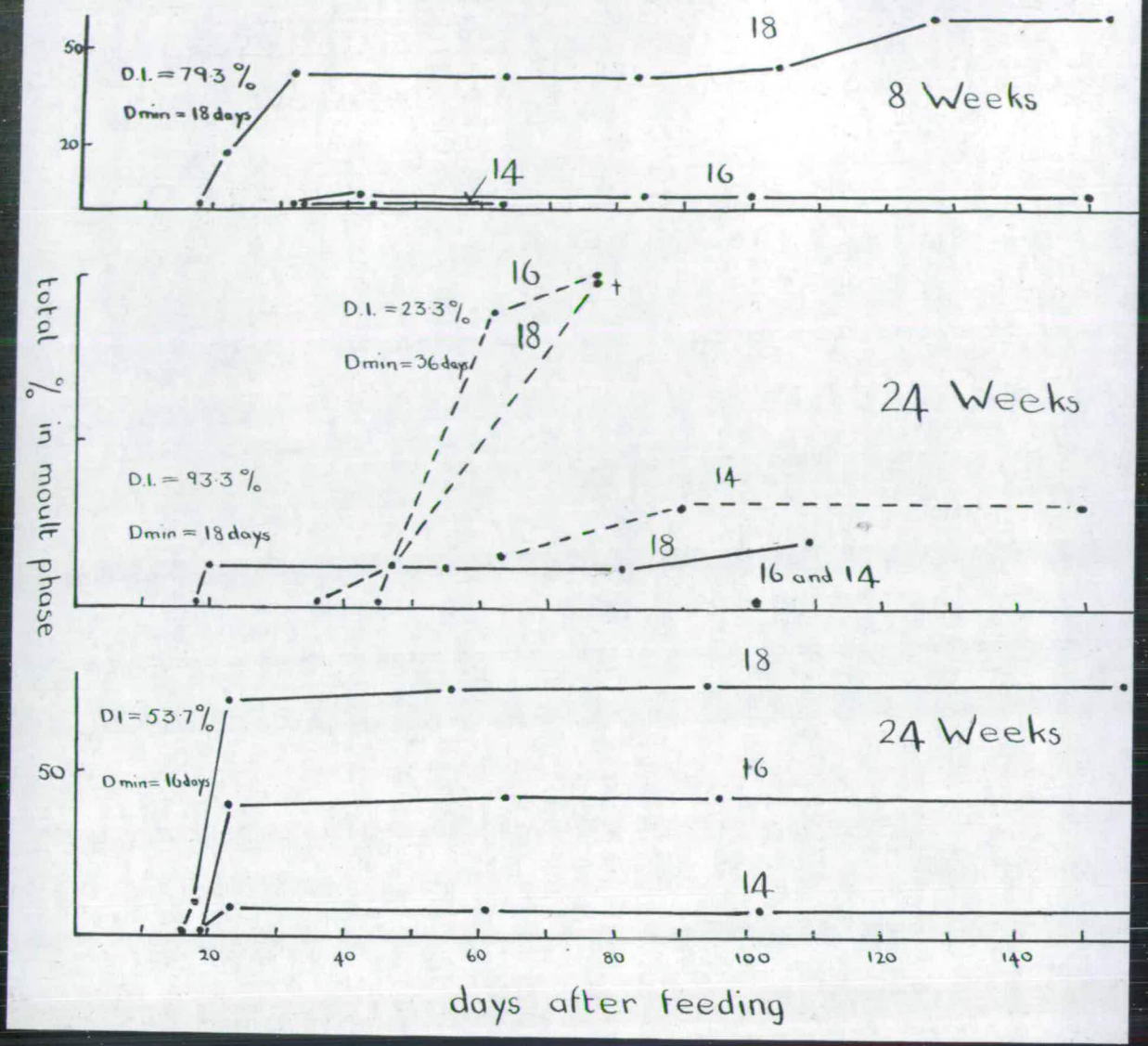


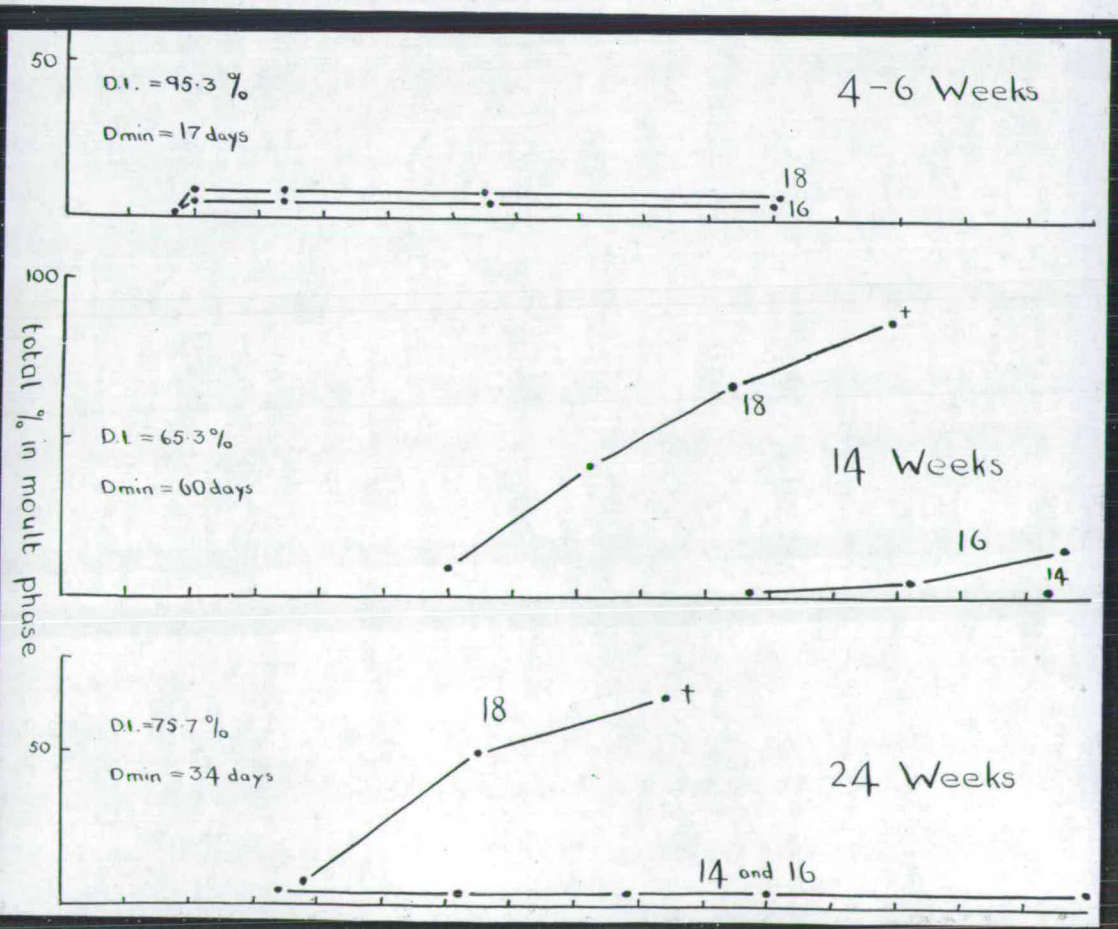
Figure 24. Graphs 1-3. Development at 15°C of larvae conditioned before feeding at 10°C, LD 18:6, for 6 to 24 weeks.
Graphs 4-6. Development at 15°C of larvae conditioned before feeding at 10°C, LD 14:10, for 6 to 24 weeks.

Figure 25. Top and middle graphs. Development at 15°C of larvae conditioned before feeding at 18.5°C, LD 18:6 for 8 and 24 weeks. Broken line graph. Development of larvae conditioned at 18.5°C, LD 12:12 for 24 weeks.

Bottom graph shows the lower incidence of diapause in some of the larval populations derived from ticks reared in the laboratory for one generation (at 15°C).

Figure 26, Development at 15°C of larvae conditioned before feeding at 23°C, LD 18:6 for 4-24 weeks.


50
20
D.I. = 79·3 %
Dmin = 18 days
18
14
16
8 Weeks
total % in moult phase
16
18
†
D.I. = 23·3 %
Dmin = 36 days
24 Weeks
D.I. = 93·3 %
Dmin = 18 days
14
18
16 and 14
50
D.I = 53·7 %
Dmin = 16 days
18
16
14
24 Weeks
20
40
60
80
100
120
140
days after feeding


50
D.I. = 95·3 %
Dmin = 17 days
18
16
4-6 Weeks
100
total % in moult phase
D.I. = 65·3 %
Dmin = 60 days
18
†
16
14
14 Weeks
50
D.I. = 75·7 %
Dmin = 34 days
18
†
14 and 16
24 Weeks

substantially the same after long day conditioning at 15°C, 18.5°C or 23°C. Larvae at the higher temperatures were ready to feed earlier than at 15°C, and different spring populations have been fed only 3 to 4 weeks after emerging. Diapause incidence was lower but never less than 46%. First generation laboratory larvae were again rather variable; the last graph in Figure 25 shows the lower incidence and short pre-moult for larvae conditioned at 18.5°C.

A short photoperiod (LD 12:12) was still effective in lowering diapause incidence even when combined with a higher temperature (18.5°C) (Figure 25, broken line graph).

It is interesting to compare the diapause incidence after prolonged conditioning at different temperature and light regimes. The combined data for larvae derived from spring and autumn adults is summarised in Figure 27, and only the results for larvae in the age groups between 12 and 24 weeks were recorded. The response superficially resembles that of short day insects, low temperatures and short days averting diapause. When the younger larvae are considered, diapause incidence is much the same under long and short days and the minimum duration of the pre-moult prolonged in the latter. Clearly the response in I. ricinus is not strictly analogous with short day insects.

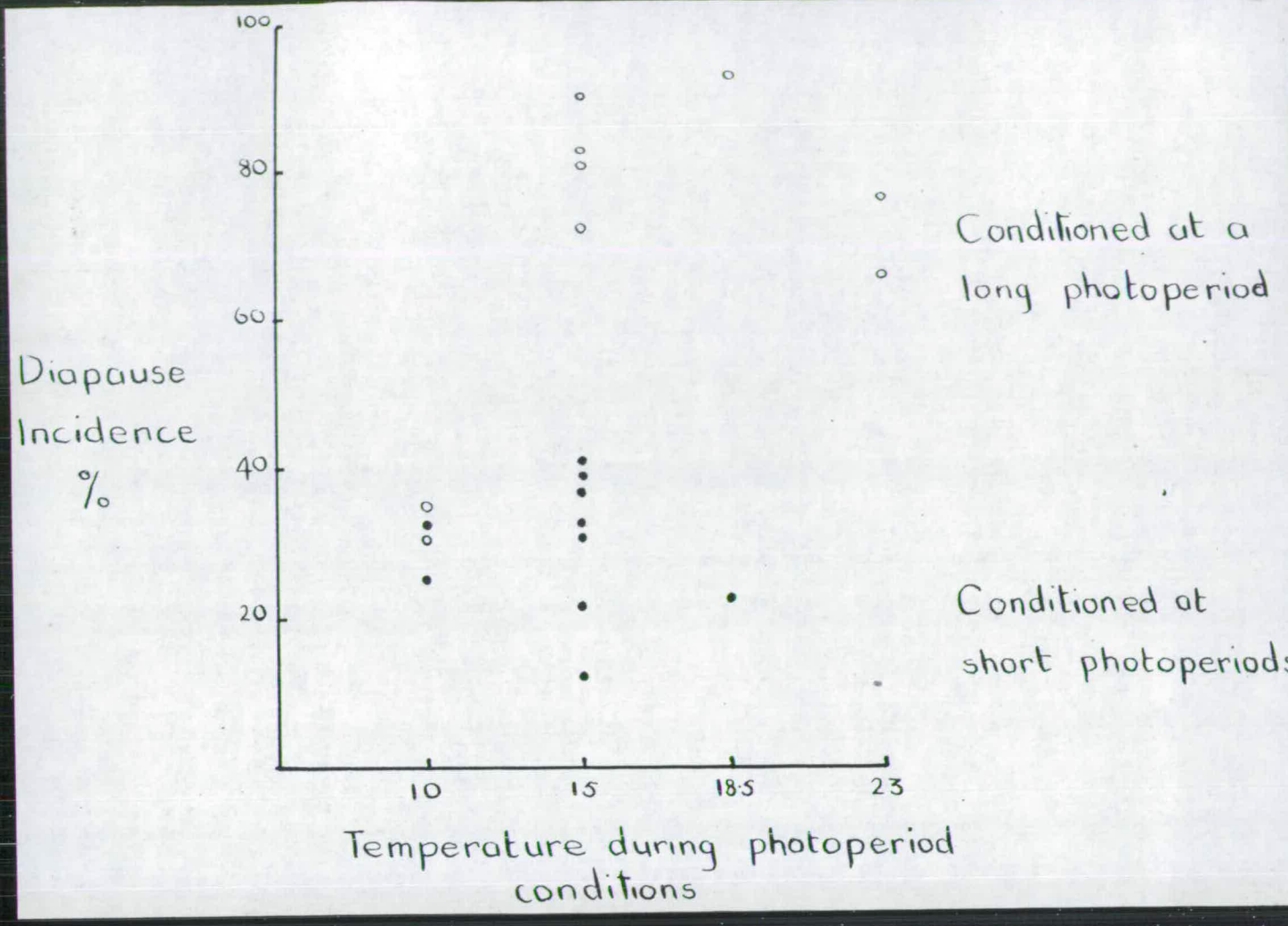
Low temperatures also prevented diapause induction in the nymphs. At 10°C nymphs were difficult to feed 4 to 8 weeks after emergence, and in many cases they were deformed or failed to break out of the old larval cuticle. An accumulation of

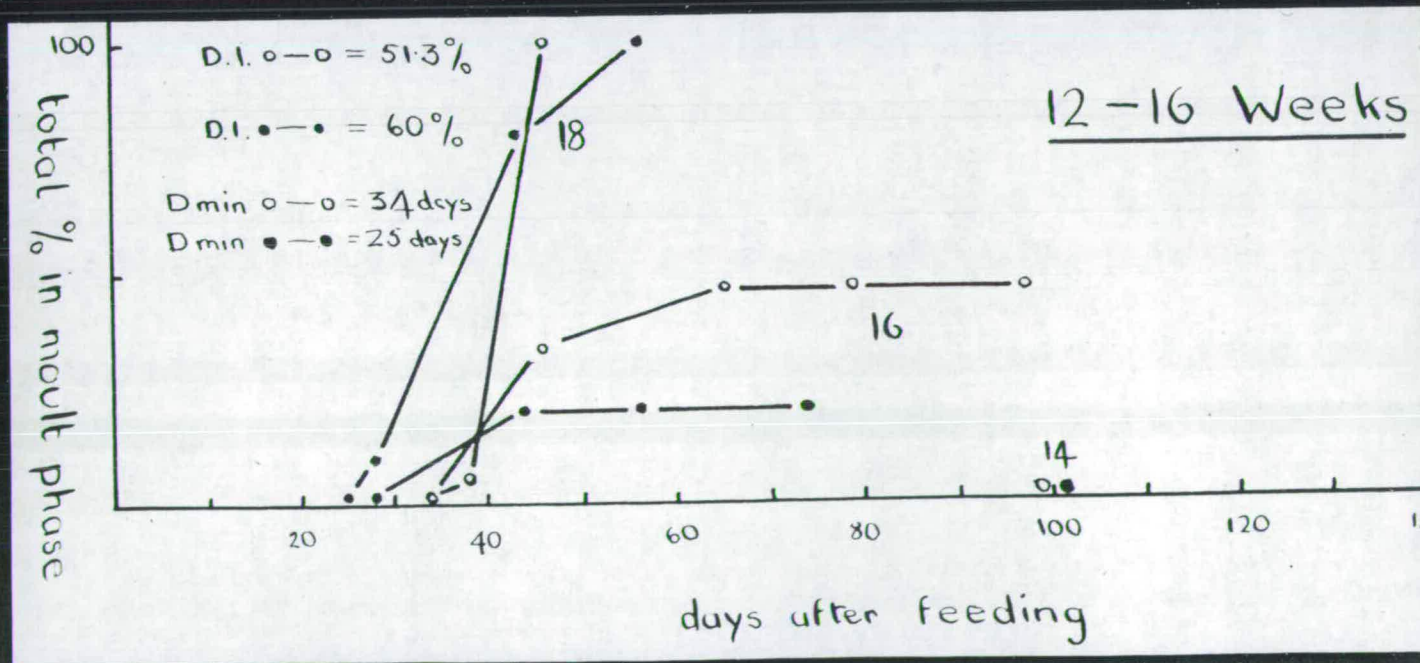
/12-16 week old

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Figure 27. A summary of the temperature and photoperiodic relations of larvae derived from spring and autumn females. The larvae were conditioned before feeding at 10°C, 15°C, 18.5°C and 23°C and at both long (open circles) and short photoperiods (closed circles). 12 to 14 week old larvae.

Figure 28. Development at 15°C of nymphs conditioned before feeding for 12 to 16 weeks at 10°C and a long (LD 18:6) or short (LD 14:10) photoperiod. •—• LD 18:6, o—o LD 14:10


100
80
60
40
20
Diapause Incidence %
Conditioned at a long photoperiod
Conditioned at short photoperiods
10
15
18·5
23
Temperature during photoperiod conditions


100
total % in moult phase
D.I. o—o = 51·3%
D.I. •—• = 60%
Dmin o—o = 34 days
Dmin •—• = 25 days
18
16
14
20
40
60
80
100
120
days after feeding
12–16 Weeks

12-16 week old nymphs were eventually fed and their development at 15°C is shown in Figure 28. There were indications, for example the shorter pre-moult and higher diapause incidence after long days, that photoperiods are still recognised at 10°C. The low temperature was clearly of overriding importance.

A few experiments at higher temperatures indicated little change in the response found at 15°C (Table 14). A short photoperiod LD 12:12 at 18.5°C effectively lowered the proportion in absolute diapause. The percentage in diapause was higher than after short days at 15°C (Figure 23), but the nymphs were derived from a different population and such comparisons were unreliable.

Table 14. Development of engorged nymphs at 15°C. The nymphs were derived from spring field larvae and were conditioned at 18.5°C and 23°C.

PRE-FEEDING EXPERIENCE	NUMBER	TOTAL % IN MOULT PHASE AFTER 200 DAYS AT 15°C LD 18:6	MINIMUM DURATION OF THE PRE-MOULT IN DAYS
4 weeks at 18.5°C LD 18:6	23	0	-
6 " " " " "	25	12	156
24 " " " " "	23	17	168
24 weeks at 23°C LD 18:6	22	13	71
24 weeks at 18.5°C LD 12:12	22	68	35

These experiments were not conducted at a suitable temperature to indicate an increase in diapause incidence with experience of higher temperatures or longer exposures to an 18 hour photoperiod. The diapause phenomenon appears to increase in intensity with continuous laboratory rearing under these conditions. Loew (1964) kept all stages of I. ricinus at room temperatures and long photoperiods, and he noticed over the

/first three

first three generations that there was a "progressive physiological weakening of the ticks". This was expressed by a "höhren Absterberate während und nach dem Saugakt, in einer grösseren zeitlichen Streuung des Schlüpftermines und in einem sich allmählich steigernden Prozentsatz der überlagernden Stadien". (Loew 1964, page 11). In a Scottish population of this tick earlier results showed that given long day conditions (LD 18:6) absolute diapause in autumn nymphs was prevented at a temperature of 18.5°C. After rearing ticks continuously at 23°C and LD 18:6 in experiments similar to Loew's, the majority of first generation nymphs (70% in a population of 146 nymphs) moulted to adults. Both the first and second generations were fed one month after emerging, but in the second generation only 26.3% from a total of 114 nymphs moulted to adults. When the temperature was raised to 25°C a farther 21% emerged but many were still in the pre-moult 65 days after the rise in temperature and all eventually died in this stage. Some of the adults fed successfully but either died before ovipositing or laid egg batches from which few larvae emerged.

High temperatures and a long photoperiod were suitable for breaking diapause in engorged ticks, but the cumulative experience increased diapause intensity until the majority of nymphs were in absolute diapause even at 25°C and a long photoperiod.

The combined attributes of May collected ticks, low diapause incidence, short pre-moult and a low limiting temperature, have not been reproduced in the laboratory experiments at

/biokinetic

biokinetic temperatures. Several authors have found that the winter experience of unfed ticks lowered the intensity of diapause, and it has generally been assumed that chilling was responsible for the absence of diapause in spring (Campbell 1948, Kheisin 1954). Joyner, Davies and Kendall (1963) found some improvement in the laboratory rearing of I. ricinus after conditioning flat ticks at low temperatures.

Chilling flat ticks at 2-5°C in the laboratory was a close approximation to winter conditions. In the field a mean temperature between 0°C to 5°C was found under vegetation for 2 to 4½ winter months during 5 years of recording (Campbell 1948).

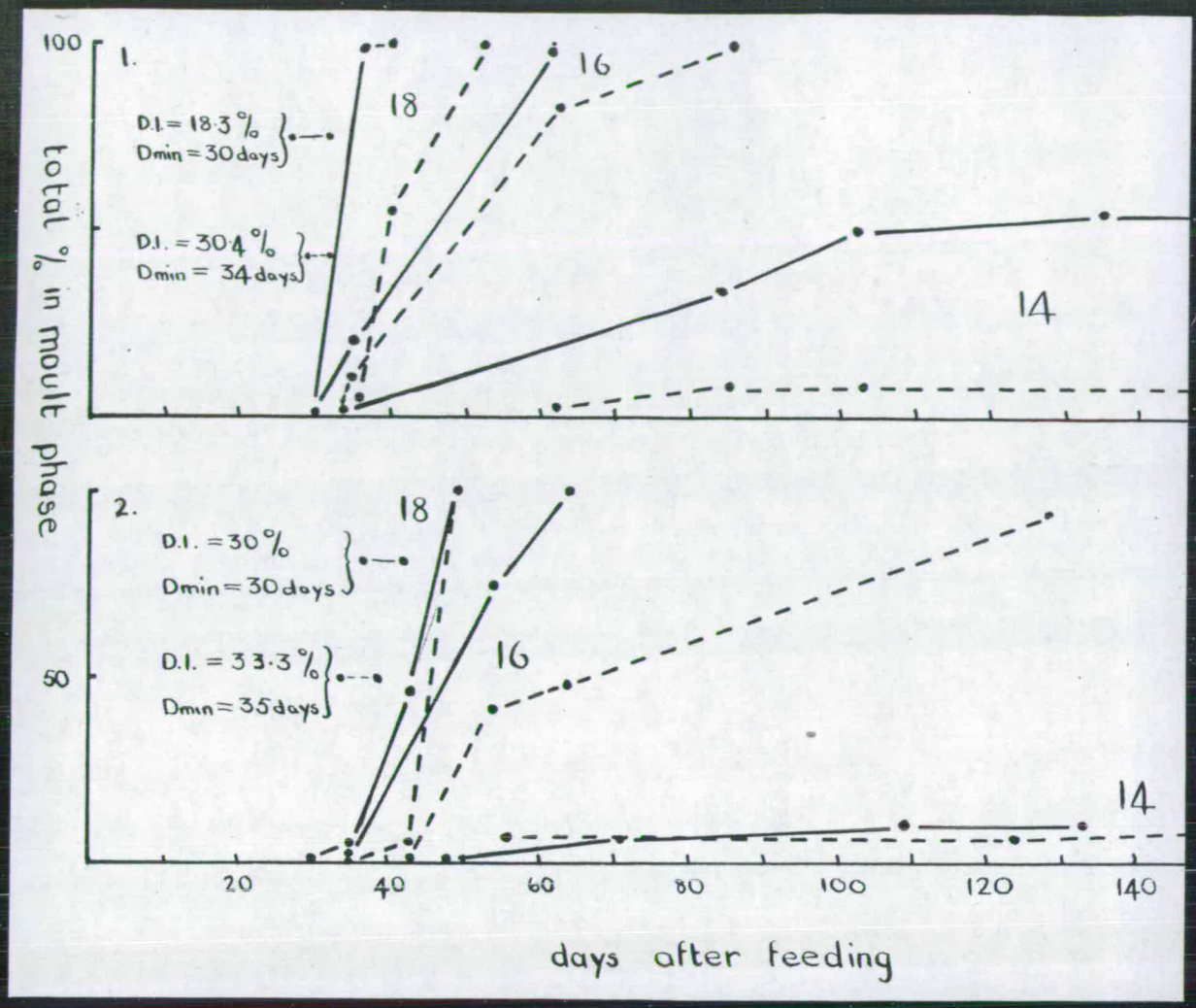
3. Elimination of diapause after conditioning unfed larvae and nymphs at low temperatures and short photoperiods followed by higher temperatures and long photoperiods.

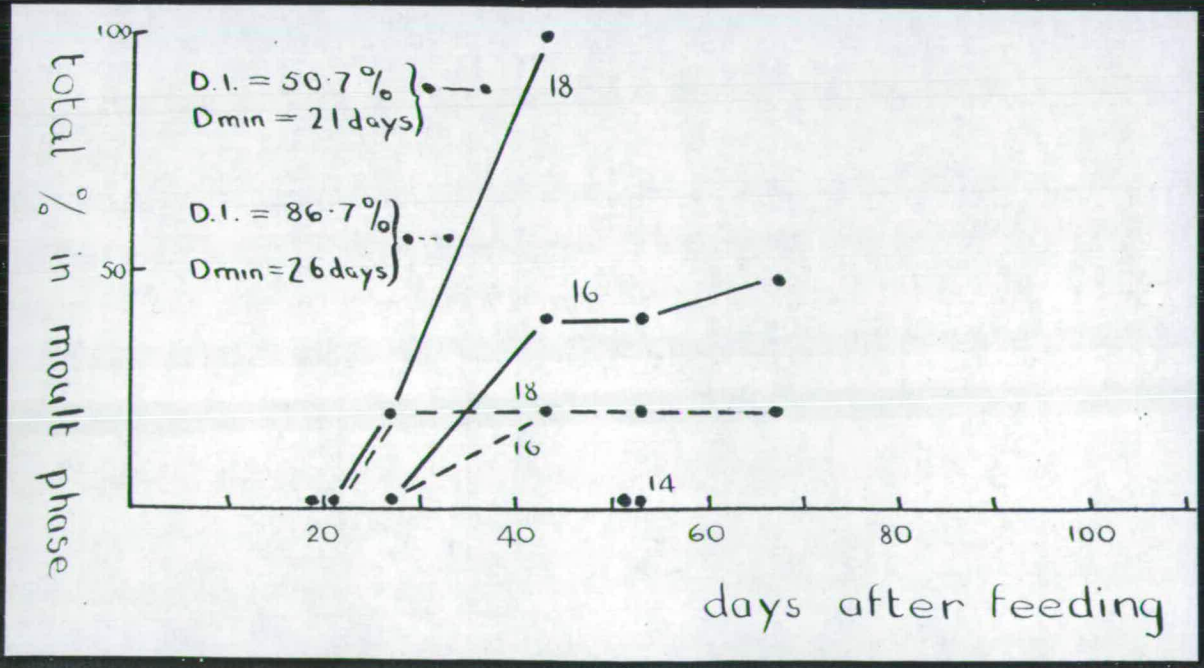
Chilling hungry larvae and nymphs at 2-5°C in the dark was slightly more effective than short photoperiods in lowering diapause incidence. Larvae and nymphs of spring origin were kept for 4 weeks at 15°C and a short photoperiod LD 14:10, half of each population were then chilled and the rest were left in short days. Larvae were chilled for 12 weeks, nymphs for 20 weeks and were then fed together with their respective short-day groups. Chilled ticks were warmed for a few hours at 15°C to recover from chill torpor, and then put on the host. Diapause incidence was low after chilling (Figure 29) and slightly lower than in the ticks conditioned at short days (broken line graphs). The minimum duration of the pre-moult in larvae and

/nymphs

Figure 29. Graph 1. Development at 15°C of larvae conditioned before feeding for 16 weeks at 15°C, LD 14:10 (broken line graph). Larvae of the same population experienced 4 weeks at 15°C LD 14:10 before being chilled for 12 weeks at 2-5°C in the dark (full line graph).
Graph 2. In similaar experiments unfed nymphs experienced 24 weeks at 15°C and LD 14:10 (broken line) or only 4 weeks followed by 20 weeks at 2-5°C in the dark (full line) before feeding.

Figure 30. Development at 15°C of nymphs which experienced before feeding 6 weeks at 15°C, LD 6:18 followed by 5 weeks at LD 18:6 (broken line graph). Other nymphs of the same population experienced 12 weeks of the short day conditions followed by 5 weeks of the long (full line graph).


100
50
total % in moult phase
1.
D.I. = 18·3 %
Dmin = 30 days
D.I. = 30·4 %
Dmin = 34 days
18
16
14
2.
D.I. = 30 %
Dmin = 30 days
D.I. = 33·3 %
Dmin = 35 days
18
16
14
20
40
60
80
100
120
140
days after feeding


100
50
total % in moult phase
D.I. = 50·7 %
Dmin = 21 days
D.I. = 86·7 %
Dmin = 26 days
18
16
18
16
14
20
40
60
80
100
days after feeding

nymphs chilled for up to 20 weeks was never less than 28 days.

Chilling alone does not account for all the characteristics of spring development. In the laboratory a close approximation was found only when prolonged conditioning at low temperatures or short days was followed by relatively brief exposure of the flat ticks to long days at 15°C and above. In an experiment with nymphs, a spring population was conditioned at a 6 hour photoperiod for 6 and 12 weeks and then kept at an 18 hour photoperiod for a farther 5 weeks before feeding. The minimum duration of the pre-moult phase (21 days) was less than expected from short day conditioning (minimum 29 days) (Figure 30). The diapause incidence was lower after the longer conditioning at a short photoperiod.

Conditioning at low temperatures followed by a long photoperiod gave a similar result.

The time required at lower temperatures has not been critically determined, but as in the previous experiment a few weeks were not sufficient. Nymphs emerging from spring larvae at 15°C were put to 2-5°C in the dark for 6 weeks, followed by 5 weeks at 15°C and a long photoperiod (LD 18:6). No marked improvement in development was found when compared with nymphs which had never been chilled. (Section IV,1). After feeding none entered the moult phase in less than 80 days at 15°C.

If conditioning was prolonged, 2-5°C in the dark was more effective than darkness at higher temperatures. Newly emerged nymphs at 15°C and an 18 hour photoperiod were put to 15°C, 10°C and 2-5°C in the dark for 20 weeks, and then returned to

/the original

the original conditions for 5 weeks before feeding. Diapause incidence and duration of the pre-moult declined progressively with the decline in conditioning temperature from 15°C to 2-5°C (Figure 31).

In a direct comparison of chilling with conditioning at a short photoperiod, the former was only slightly more advantageous. Newly emerging nymphs were chilled for 16 weeks at 2-5°C in darkness or kept at 15°C and a 6 hour photoperiod. Both groups then experienced 6 weeks at 15°C and 18 hours of light per day before feeding. Diapause incidence was lower in the previously chilled ticks, 21.7% compared with 35%, and the pre-moult shorter, minimum 16 compared with 18 days (Figure 31, last graph).

In the chilled nymphs diapause incidence and duration of the pre-moult were comparable with the lowest levels found in spring field ticks (Figures 2 and 3).

For the larvae, earlier results (Section IV, 1.) indicated that conditioning prior to experience of long days was not essential when post-feeding development proceeds at 15°C. Diapause incidence was relatively low (44.7%) and the minimum pre-moult short (20 days) in some larval populations experiencing only 4 weeks of long days prior to feeding. In the more limiting conditions imposed by a lower incubation temperature (10°C) these larvae failed to enter the moult phase. When comparing pre-feed conditioning at low temperatures and short photoperiods, larvae were incubated at both 15°C and 10°C

/after feeding.

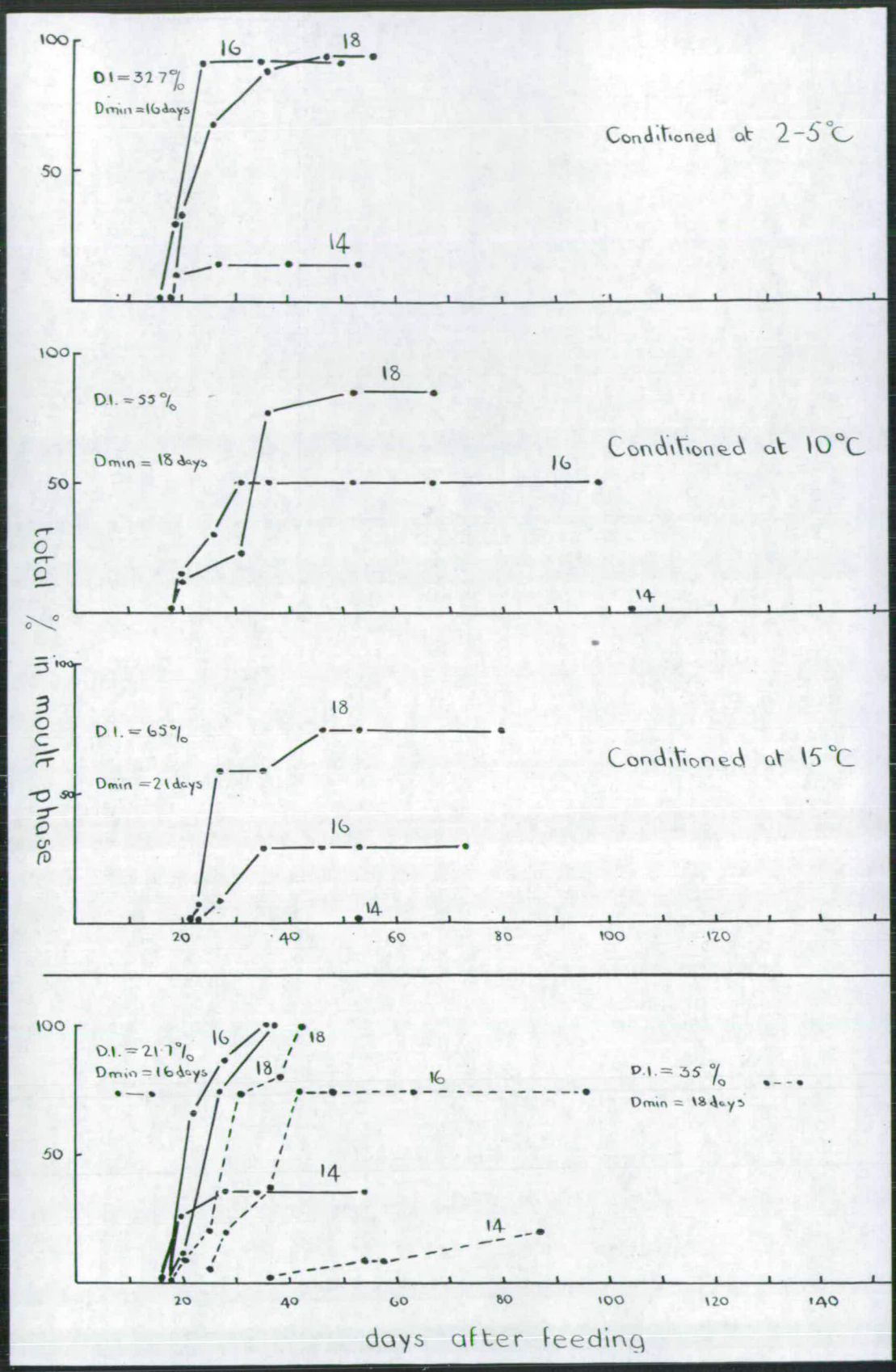


Figure 31. First three graphs : Development at 15°C of nymphs conditioned before feeding for 20 weeks at 15°C, 10°C or 2-5°C in the dark followed by 5 weeks at 15°C LD 18:6.

Last graph :Development at 15°C of nymphs conditioned before feeding for 16 weeks at either 2-5°C in the dark (full line graph) or 15°C LD 6:18 (broken line graph). Both groups then experienced 6 weeks at 15°C LD 18:6 before feeding.

after feeding.

If experience of long days was postponed until immediately after the larvae emerged, diapause incidence was lower than for larvae which experienced only long days during embryonic and later development.

Larvae which were kept during embryonic development at 15°C and 6 hours of light per day were moved on emerging to an 18 hour photoperiod. Diapause incidence was lower at an incubation temperature of 15°C than for larvae of the same spring population which experience only an 18 hour photoperiod (Figure 32). Both groups were fed after 5 weeks at long days.

This experiment has been repeated with another population and gave the same result. Presumably the photoperiod is recognised by the embryo and either short days have a conditioning effect prior to emergence or the rise in diapause incidence associated with increasing exposure to long days, is delayed. Both factors could be operating.

At a lower post-feeding temperature (10°C), none of the larvae which experienced short days in the egg, developed in less than 180 days. Incubation at this temperature demonstrated the advantage of prolonged pre-feed conditioning at low temperatures or short days. Larvae which had recently emerged were either chilled for 16 weeks or left for this time at 15°C and a 6 hour photoperiod. Following a farther 5 weeks at 15°C and long days (LD 18:6) the larvae were fed and incubated at 10°C and 15°C. The duration of the pre-moult was shorter for the chilled ticks, but the proportion in diapause was only less at 10°C. (Figure 32). Engorged larvae at 10°C and 18 hours of light per day had, considering the temperature,

/a short

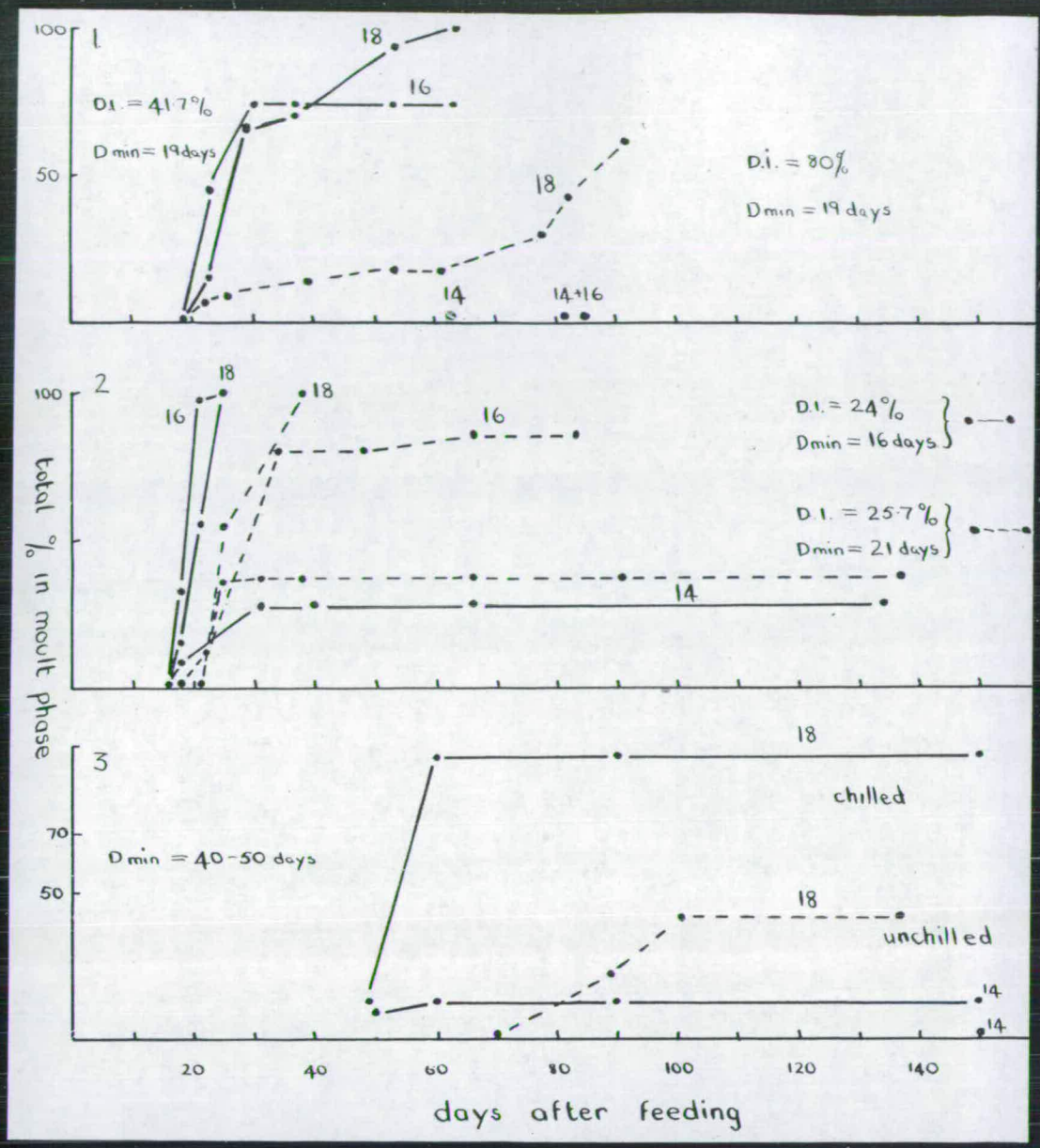


Figure 32. Graph 1. Development at 15°C of larvae conditioned before feeding for 5 weeks at 15°C LD 18:6 (broken line graph). Larvae of the same population emerged in short days (0 to 3 weeks at 15°C LD 6:18), then experienced 5 weeks at 15°C LD 18:6 for 5 weeks before feeding (full line graph).
Graph 2. Development at 15°C of larvae conditioned before feedimg for 16 weeks at either 15°C LD 6:18 (brokem line graph) or 2-5°C in the dark (full line graph). These larvae then experienced 5 weeks at 15°C LD 18:6 before feeding.
Graph 3. Development at a post-feeding temperature of 10°C of the same larvae shown in Graph 2.

a short pre-moult (between 40-50 days) and only 6% in diapause. The unchilled larvae (broken line graph) developed less readily at these conditions.

The nymphs differ from the larvae in the greater requirement for conditioning if development at a post-feeding temperature of 15°C is to proceed with the minimum expression of diapause.

Lower temperatures were important for the larvae during the conditioning period. After 16 weeks at 10°C or 2-5°C in the dark, followed by 6 weeks in long days at 15°C, the two populations were compared at 15°C and 10°C after feeding (Figure 33 graphs A and B). A temperature between 2-5°C was more effective in all respects, incidence 3.7% lower, minimum pre-moult 2 days less at 15°C and 70% more in the moult phase at the lower temperature, but as with the nymphs conditioning at 10°C was effective when post-feeding development proceeded at a higher temperature.

Part of the effect of chilling in the dark may be due to the absence of photoperiodic recognition. Larvae were conditioned for 16 weeks at 10°C and long (LD 18:6) or short (LD 14:10) photoperiod. Both groups were then kept at 15°C and an 18 hour photoperiod for 5 weeks before feeding. Diapause incidence and duration of the pre-moult were almost identical (Figure 33, graphs C and D).

In this section the importance of the conditioning period has been stressed. Only larvae and nymphs derived from spring ticks collected in Perthshire have been used and in each experi-

/ment only larvae

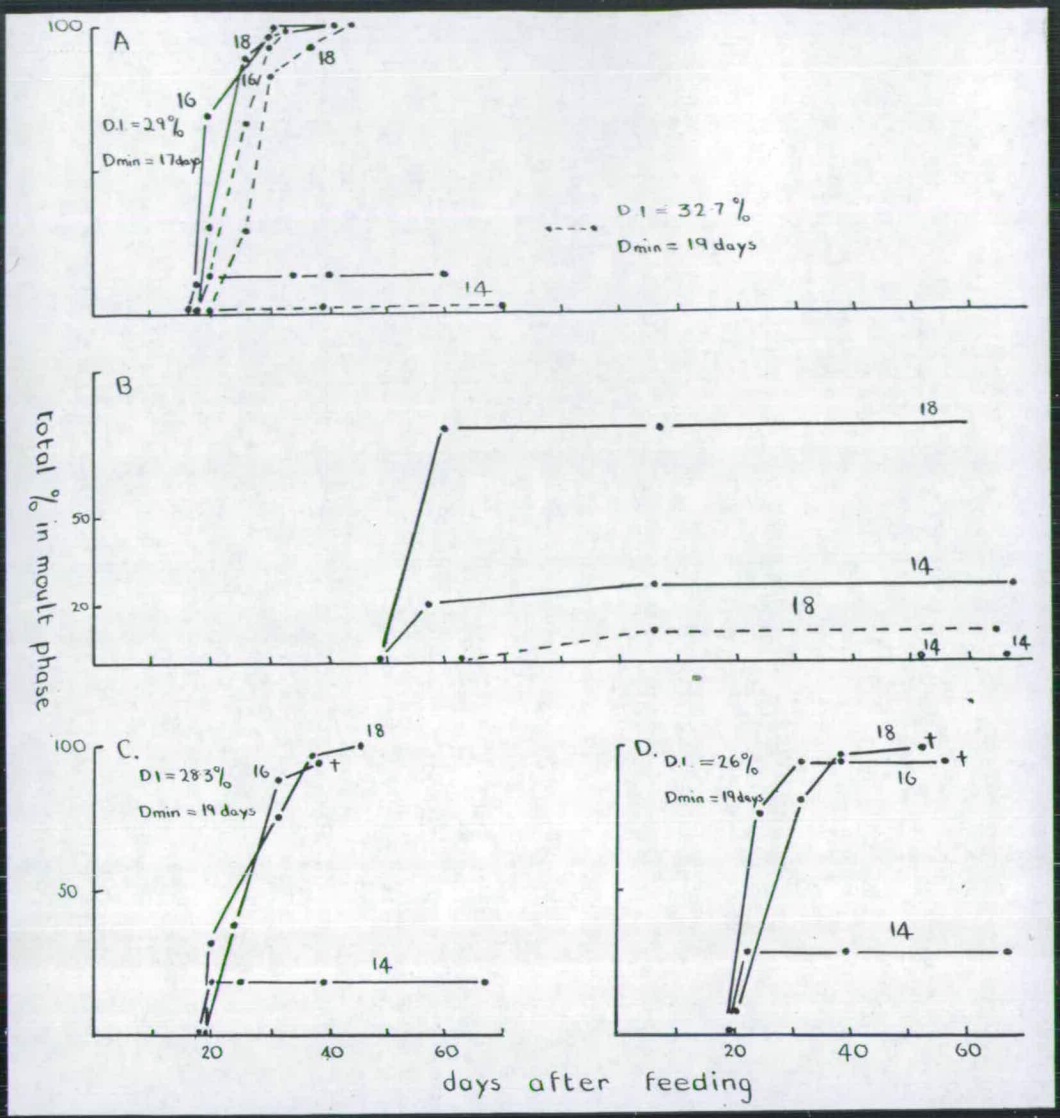


Figure 33. Graph A. Development at 15°C of larvae conditioned before feeding for 16 weeks at 10°C, LD 0:24 (broken line graph) or 2-5°C, LD 0:24 (full line graph). These larvae then experienced 6 weeks at 15°C, LD 18:6 before feeding.
Graph B. Development of these same larvae at 10°C after feeding.
Graphs C and D. Development at 15°C of larvae conditioned before feeding for 16 weeks at 10°C and an 18 hour photoperiod (C) or a 14 hour photoperiod (D). These larvae experienced 5 weeks at 15°C, LD 18:6 before feeding.

experiment only larvae from engorged females collected on the same day or nymphs from the same batch of larvae have been compared. The relatively brief experience of long days after the prolonged conditioning period was always between 4 to 6 weeks and in each experiment always of the same duration in the different groups. There is a progression in the long day reaction after chilling, and a better method of comparing the different types of conditioning would be to feed ticks after increasing experience of long days.

This progression has only been determined after conditioning at 2-5°C for 14 weeks. Larvae were fed directly after chilling and in a series after 2, 4, 10 and 14 weeks at 15°C and an 18 hour photoperiod. Diapause incidence and duration of the pre-moult declined from 0 to 4 weeks experience of long days. Thereafter the minimum duration (18 days) remained constant but the incidence increased, particularly after 10 to 14 weeks exposure (Figure 34).

Chilled nymphs were fed after 2, 6, 10 and 14 weeks at 15°C and 18 hours of light per day. (Figure 35). The pre-moult was short and incidence of diapause lower with an increase from 2 to 6 weeks at long days. Diapause was already strongly manifest after 10 weeks, an earlier increase in diapause than was found for the larvae.

Long days after chilling not only reduced the pre-moult to a minimum, but diapause incidence was also lowered. These improvements after chilling result from recognition of long days above 10°C, rather than a response to the increase in

/temperature.

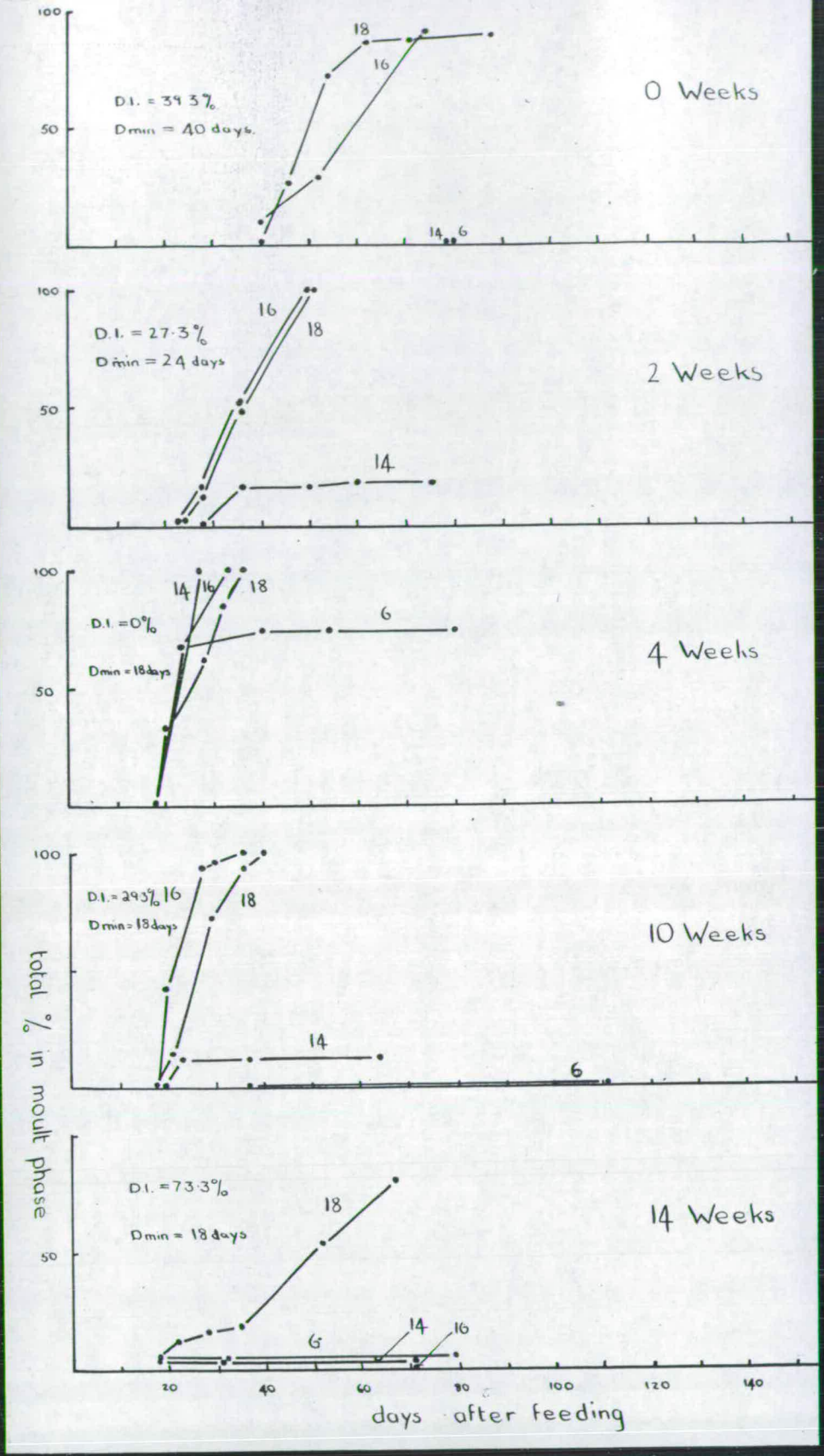


Figure 34. Development at 15°C of larvae conditioned before feeding for 14 weeks at 2-5°C in the dark followed by 0 to 14 weeks at 15°C, LD 18:6.

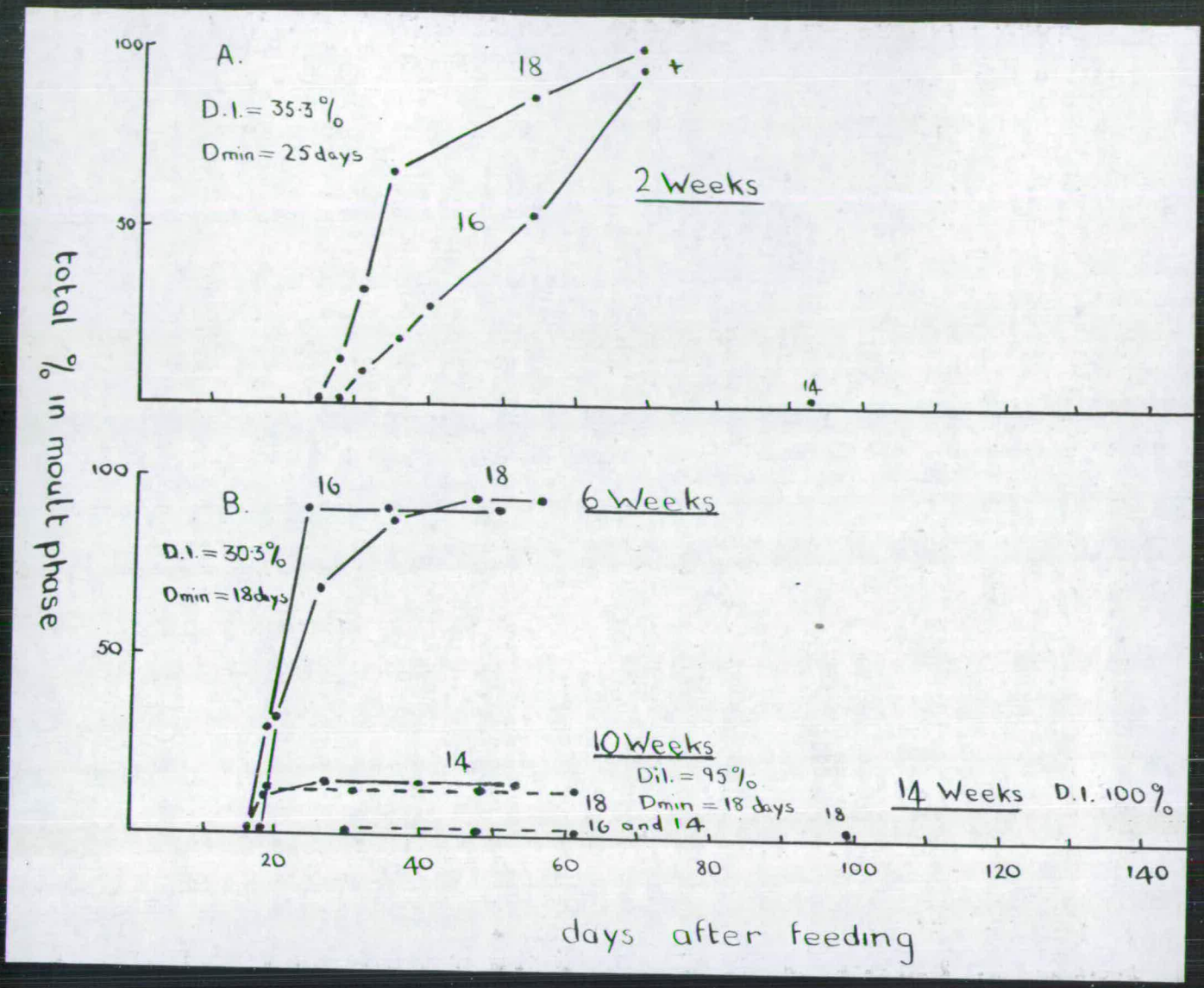


Figure 35. Development at 15°C of nymphs conditioned before feeding for 16 weeks at 2-5°C in the dark followed by 2, 6, 10 and 14 weeks at 15°C, LD 18:6.

temperature.

Considering firstly the duration of the pre-moult after experience of long days at 10°C, 15°C and 23°C. In several experiments with larvae of different populations kept at these conditions the ticks with the shortest pre-moult were compared (Figure 36, graph 1). The duration of exposure after chilling was 3 weeks at 23°C and 4 weeks at 10°C and 15°C. There was a marked decrease in duration of the pre-moult as the temperature experienced during long days rose from 10°C to 15°C, but little farther change with a rise in temperature to 23°C. After a short photoperiod (LD 14:10) at 15°C, and a long (LD 18:6) or short (LD 14:10) photoperiod at 10°C, the pre-moult was always delayed (Figure 36, graphs 1 and 2).

The diapause incidence was much the same in different chilled populations which experience long days at different temperatures. Larvae which showed the lowest diapause incidence were exposed to an 18 hour photoperiod at 10°C, 15°C, 18.5°C and 23°C for 8, 4, 4 and 3 weeks respectively. After feeding, incidence at 15°C was 20.1%, 0%, 12.7% and 16.7% respectively. The 15°C and 23°C larvae were also incubated at 10°C and LD 18:6 after feeding, and 8% and 23% were in diapause. It must be admitted that when larvae of mixed origin have been used the results are not always reliable. Also, a complete series of larvae at different ages have not been obtained at post-chilling temperatures other than 15°C. These results nevertheless suggest that temperatures of 18.5°C and 23°C were no more effective than 15°C.

/The return

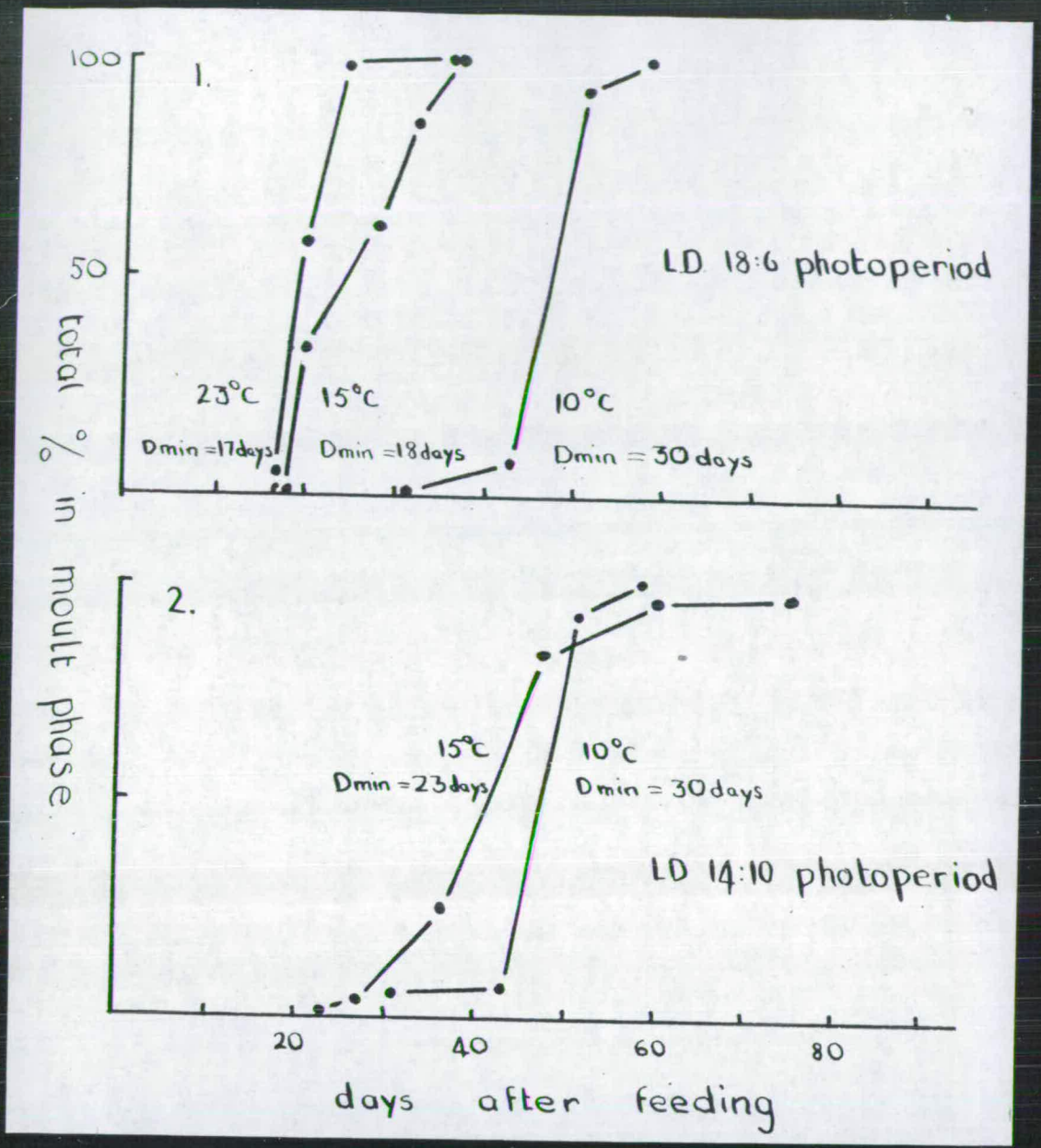


Figure 36. The minimum duration of the pre-moult at 15°C, LD 18:6. Larvae were conditioned before feeding for 16 weeks at 2°5°C in the dark. The larvae in Graph 1. then experienced an 18 hour photoperiod for 3 weeks at 23°C or 4 weeks at 15°C or 10°C before feeding. Larvae in Graph 2. experienced a 14 hour photoperiod for 4 weeks at 15°C or 10°C.

The return of intense diapause after 10 to 14 weeks experience of long days was not found under short day conditions. The response to short photoperiods was not greatly affected by the previous chilling experience, with an increase from 6 to 14 weeks at 15°C and LD 14:10, diapause incidence in nymphs rose by only 20% (Figure 37). Larvae had a low percentage in diapause, even at 10°C, when their pre-feeding history included chilling followed by 4 or 14 weeks at a 6 hour photoperiod (Figure 38, broken line graphs). Larvae which experienced 14 weeks at long days were, as expected, in diapause (Figure 38).

After chilling, 23°C and 18°C were no more effective than 15°C, but at these higher temperatures nymphal diapause supervened 2 to 4 weeks earlier. In a chilled population of nymphs fed after 4, 6 and 8 weeks, at 23°C and LD 18:6, the incidence of diapause was 33.3%, 88.3% and 100% respectively. At least 10 weeks of long days at 15°C were necessary to raise diapause incidence to these levels. The progression in the long days response is apparently completed more rapidly at high temperatures. At a low temperature after chilling it is doubtful if it proceeds at all, even after 14 weeks at 10°C and LD 18:6 diapause incidence was low (Figure 39).

Prior to chilling, the photoperiodic threshold in the response of unfed ticks was between 16 and 14 hours of light per day. After chilling, 6 weeks experience of a 16 or 14 hour photoperiod was compared in an identical population of nymphs. (Figure 40). The minimum duration of the pre-moult was only 19 days after the longer photoperiod and 31 days after the

/shorter.

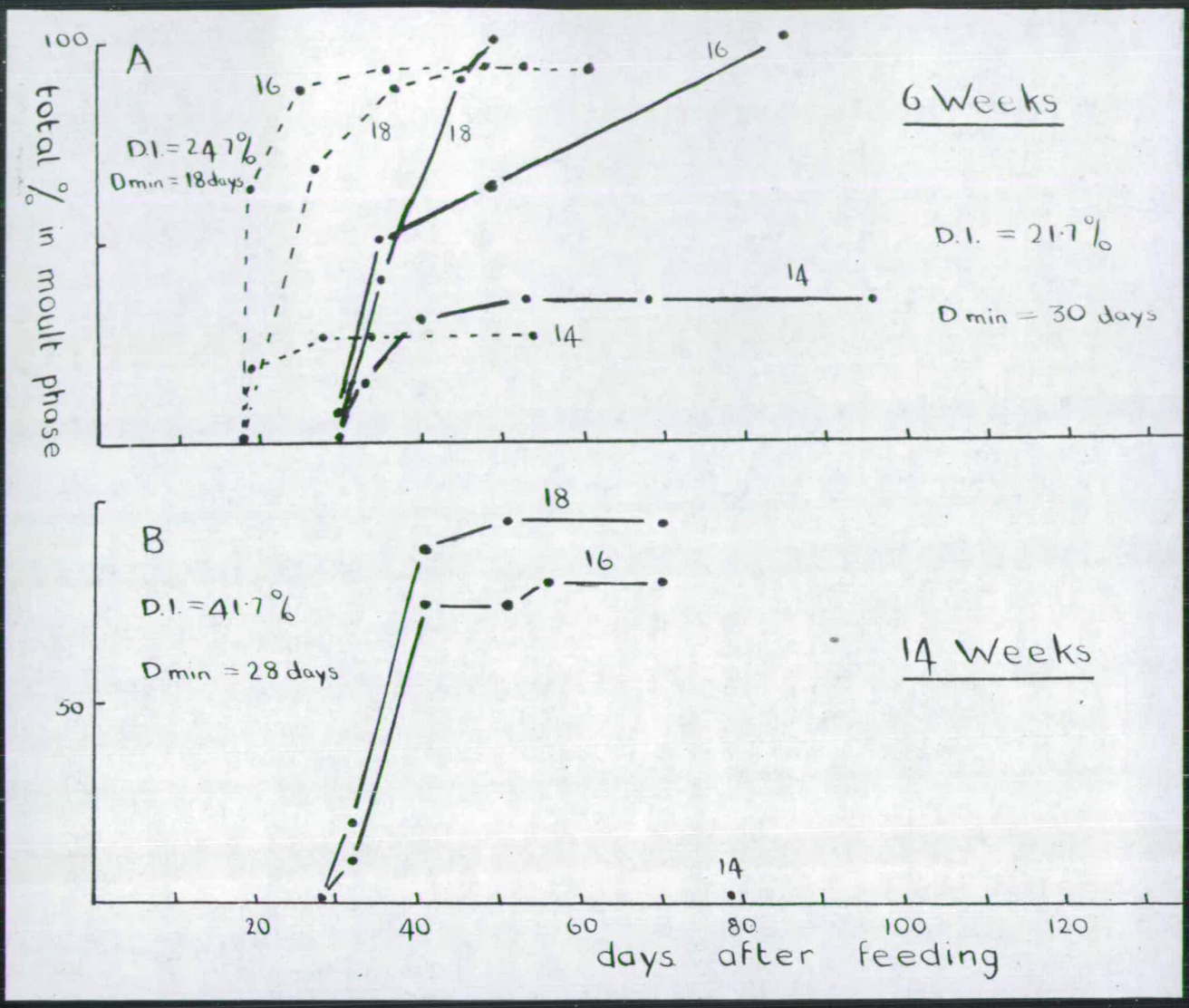
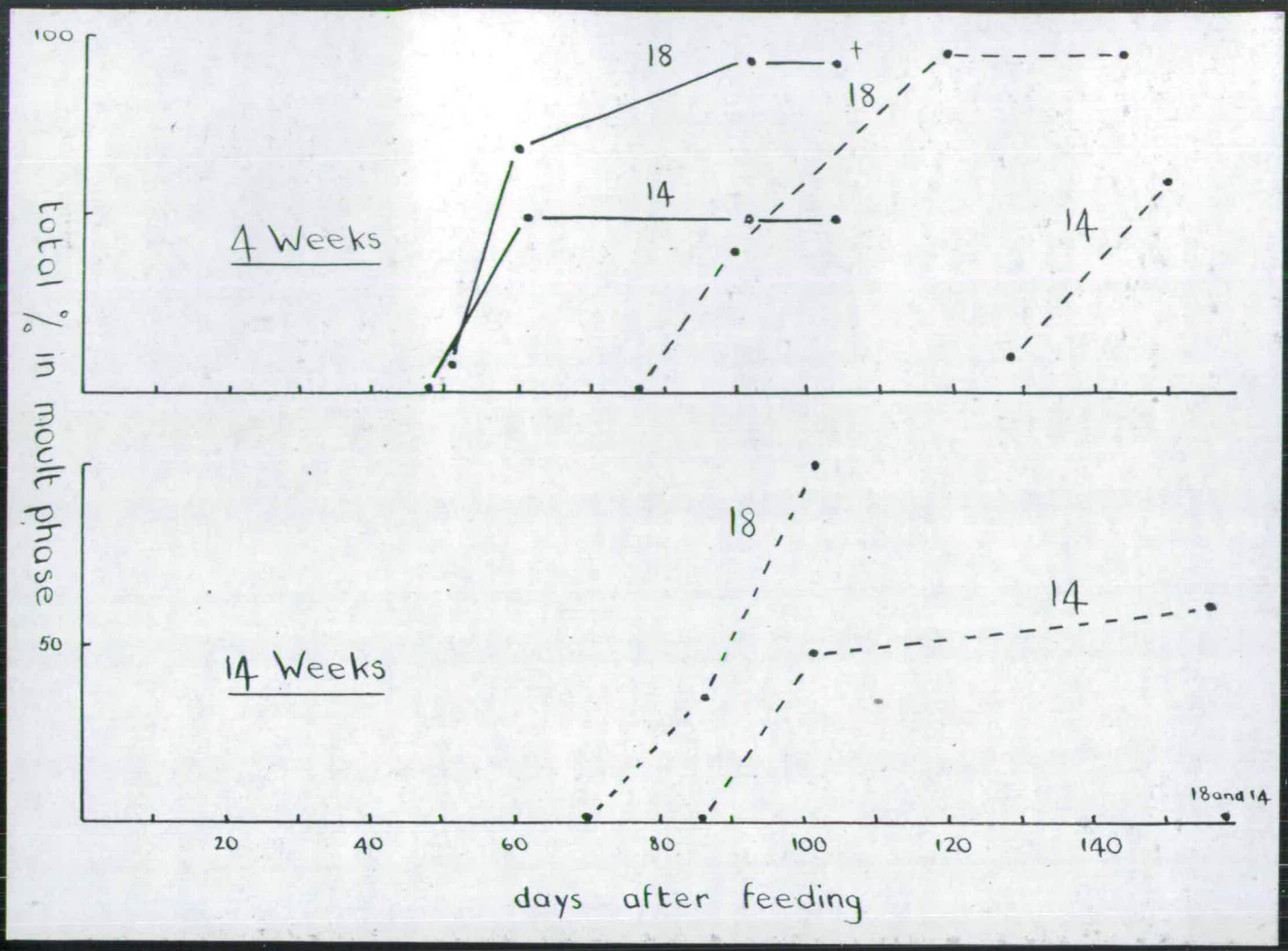
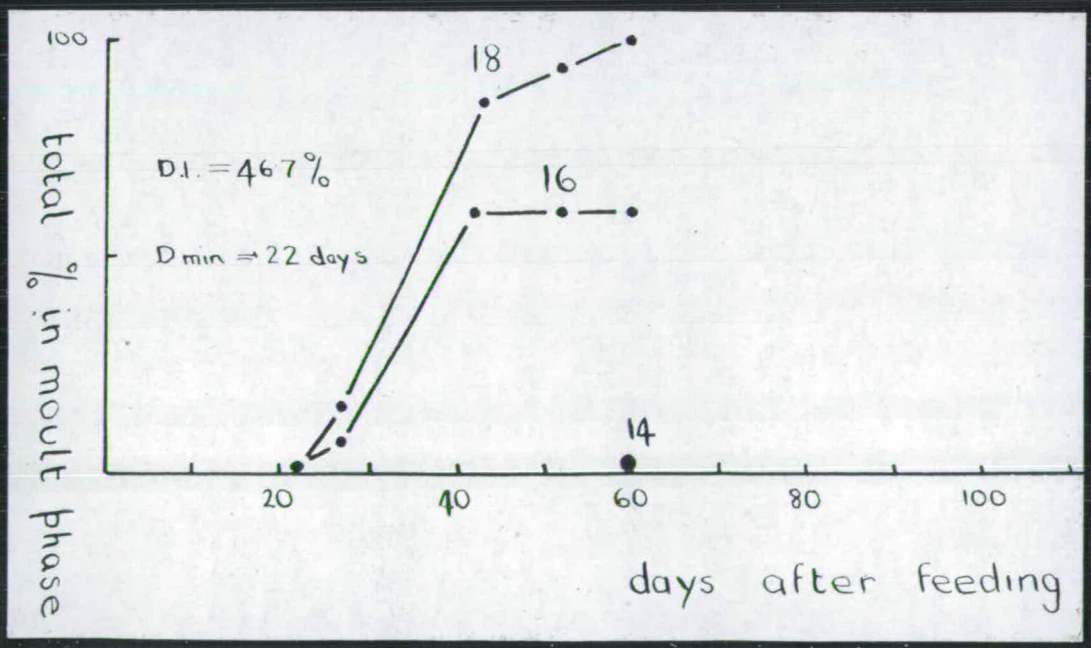


Figure 37. Development at 15°C of nymphs conditioned before feeding at 2-5°C in the dark for 14 weeks, followed by 6 weeks (Graph A) or 16 weeks (Graph B) at 15°C and LD 14:10. The broken line graph shows the development of the same populations which experienced 6 weeks at 15°C and LD 18:6 before feeding.

Figure 38. Development at 10°C of larvae conditioned before feeding for 14 weeks at 2-5°C in the dark followed by 4 or 14 weeks at 15°C and a long or short photoperiod. Development of larvae experiencing a short photoperiod (LD 6:18) is shown in broken lines. Long day larvae (LD 18:6) in full lines.

Figure 39. Development at 15°C of nymphs conditioned at 2-5°C in the dark for 16 weeks followed by 14 weeks at 10°C, LD 18:6 before feeding.


100
50
total % in moult phase
4 Weeks
18
18
14
14
†
14 Weeks
18
14
18and14
20
40
60
80
100
120
140
days after feeding


100
total % in moult phase
D.I. = 46.7%
D min = 22 days
18
16
14
20
40
60
80
100
days after feeding

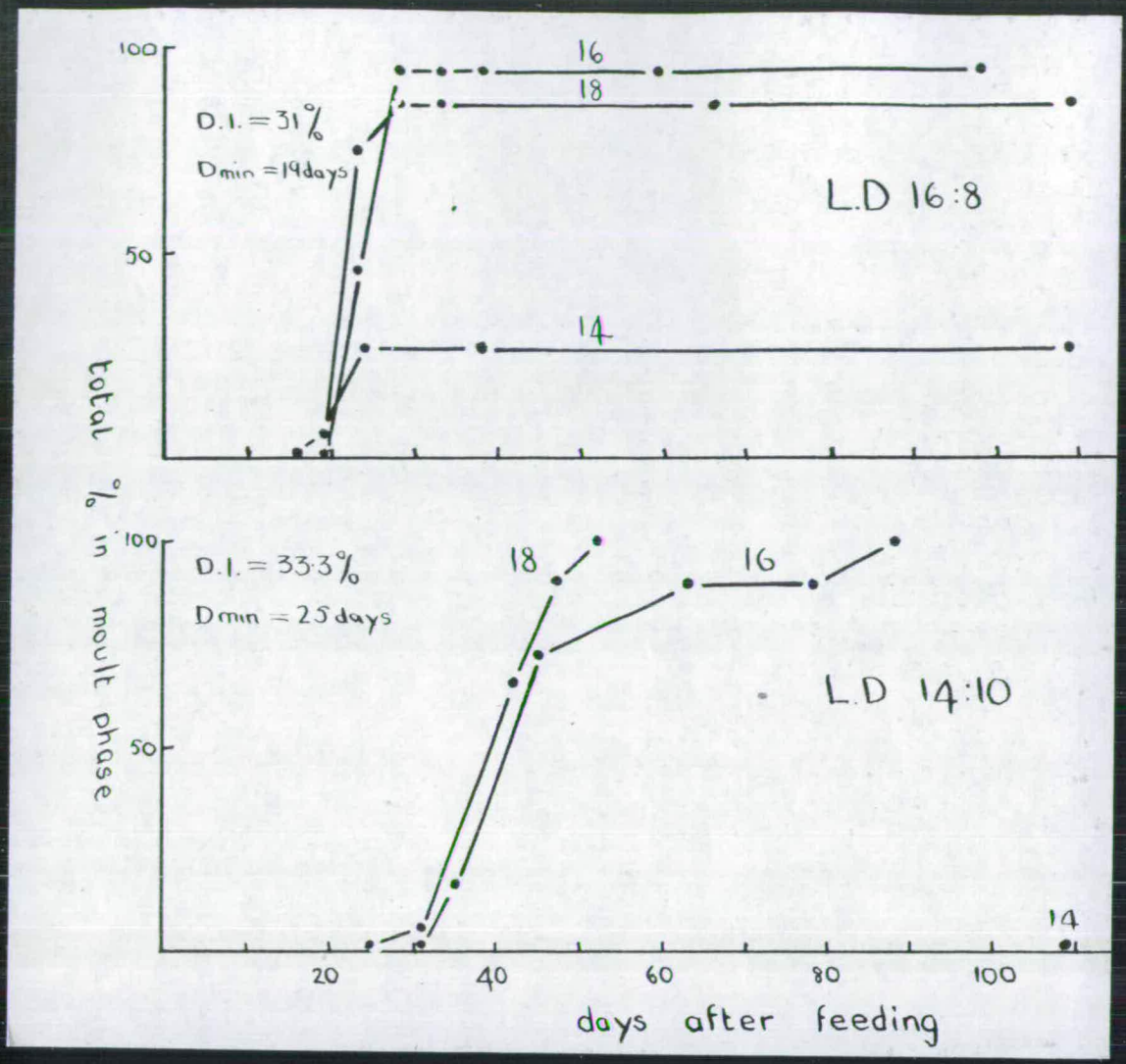


Figure 40. Development at 15°C of nymphs conditioned at 2-5°C in the dark for 14 weeks before feeding. Top graph; nymphs experienced 6 weeks at 15°C LD 16:8 between chilling and feeding. Bottom graph; nymphs experienced 6 weeks at 15°C LD 14:10 at this time.

shorter. A 16 hour photoperiod at 15°C was almost as effective as 18 hours in the reintroduction of diapause; after an increase in exposure of chilled nymphs from 6 to 10 weeks diapause incidence rose from 31.0% to 88.2%. The pre-feeding threshold photoperiod is less vulnerable to modification as a result of prior experience, although of course the response to a fixed photoperiod alters with time.

The previous sections, on laboratory reared ticks, have been concerned with the influence of pre-feeding conditions in the regulation of diapause in larvae and nymphs. Although an intense embryonic diapause is associated with the midsummer experience of hungry adults, it has been found difficult to induce diapause in the laboratory under the same conditions which were successful for larvae and nymphs. It was difficult to feed sufficient numbers of adults for these experiments. The longest delays were found in ticks which were reared for one generation or more at a high temperature (23°C) and a long photoperiod (LD 18:6).

4. Lack of response to small daily changes in the photoperiod.

One of the few arthropods which apparently responds to changing photoperiods is the tick D. variabilis. Attachment and feeding of larvae and nymphs was most successful after experience of increasing photoperiods and least successful after decreasing ones. Fixed long or short photoperiods were intermediate in effect (Smith and Cole 1941).

/Experiments

Experiments with the dragonfly Anax imperator suggest that diapause in the final instar is induced by fixed short photoperiods, or by declining photoperiods irrespective of their lengths. The absence of diapause is associated with a long or increasing photoperiod.(Corbet 1956). The results were not particularly easy to interpret, but the conclusions concerning declining photoperiods may be open to doubt. These started at 16 hours of light per day, and although this may be a long photoperiod his results suggest that an even longer one is required to avert diapause. His conclusions do not explain why larvae in early final stage (sensitive period) in the beginning of June, enter diapause. If very small daily increments can not be recognised as he has suggested, then neither declining nor short photoperiods can be responsible for diapause at this time of year.

In another insect with a prolonged sensitive stage, the red locust Nomadacris septemfasciata, a greater intensity of diapause was associated with late summer conditions. Norris (1959) suggested that declining photoperiods may be responsible. In later experiments she found the greatest intensity of diapause when fixed long photoperiods were followed by fixed short ones. Small daily changes were no more effective (Norris 1965).

I. ricinus has, in common with these species, slow development and a long sensitive stage. In the laboratory, larvae derived from the same spring stock were chilled for 16 weeks. One batch was put to 15°C and a solar photoperiod on the 2nd February (light phase 8 hours) and were not fed until the 17th

/March

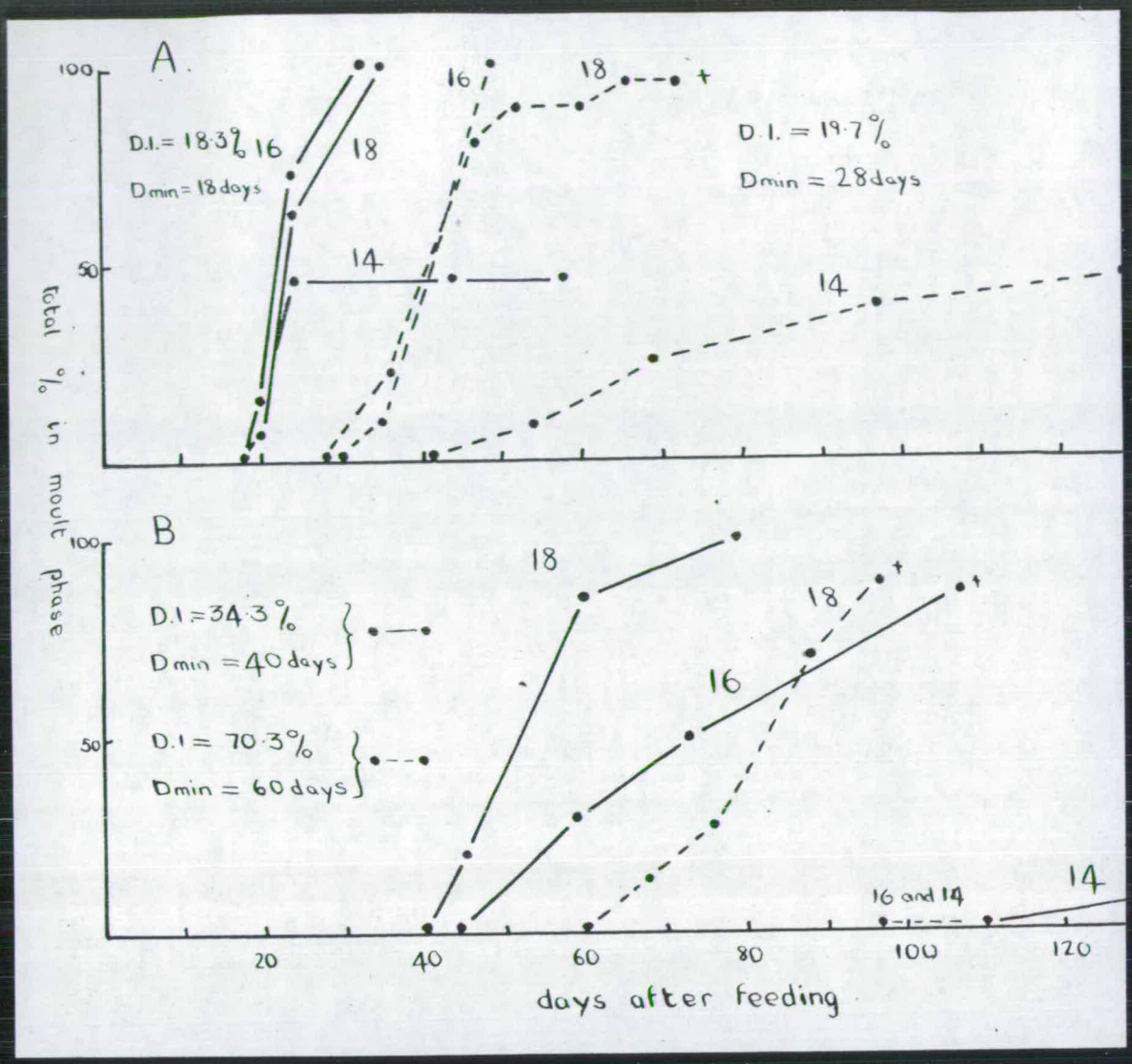


Figure 41. Graph A. Development at 15°C of larvae chilled at 2-5°C in the dark followed by experience of a photoperiod increasing by small daily increments from 8 hours to 11 hours 50 mins. (broken line graph) or from 14 hrs. 5 mins. to 16 hrs. 50 mins. (full lime graph). Graph B. Development at 15°C of larvae experiencing a photoperiod declining from 17 hours 20 mins. to 14 hrs. 15 mins. (broken line graph) or 14 hrs. to 8 hrs. 40 mins. (full line graph).

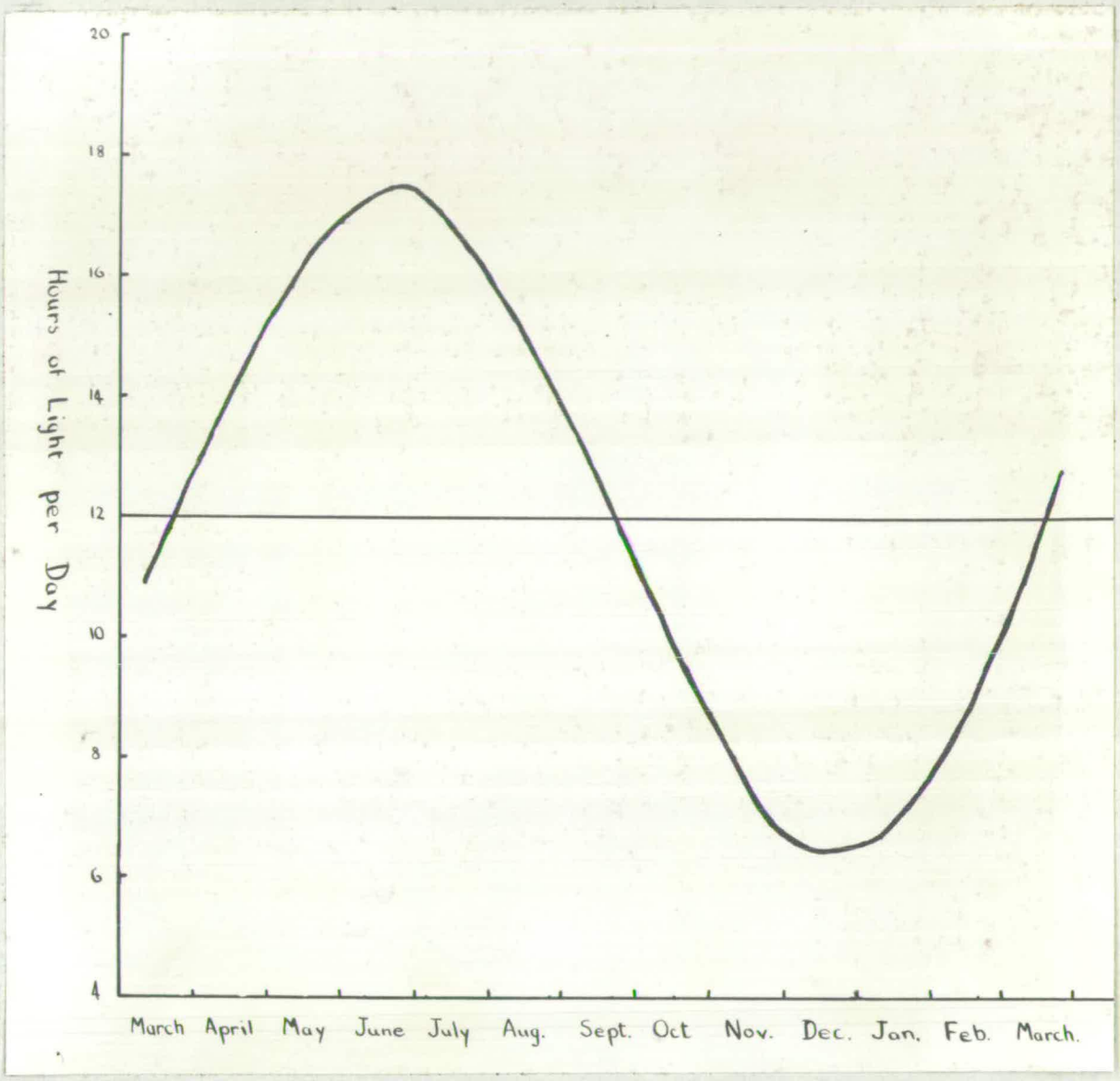


Figure 41a. The light regime used in the solar experiments is shown above. It is close to the natural photoperiod at a latitude of 57° N.

March (light phase 11 hours 50 minutes). Their development was comparable with larvae which experienced a fixed short photoperiod after chilling, their pre-moult was prolonged (28 days) (Figure 41). The second batch experienced short photoperiods starting on the 27th April (light phase 14 hours 5 minutes), followed by long photoperiods. They were fed on the 16th June (light phase 16 hours 50 minutes) and development was no different from chilled larvae which experienced fixed long days; their minimum pre-moult was only 18 days (Figure 41).

A similar lack of response was found in declining photoperiods. Chilled autumn females were put to 15°C and the solar photoperiod. The first batch of larvae did not begin to emerge until the end of June (light phase near to 17 hours 20 minutes), and they were fed 8 weeks later on the 26th August (light phase 14 hours 15 minutes). Diapause incidence was high (70.3%) but no higher than expected from prolonged exposure to a fixed long photoperiod. The second batch did not begin to hatch until the 28th August (light phase 14 hours), and the larvae were fed on the 30th October (light phase 8 hours 40 minutes). The minimum duration of the pre-moult was rather prolonged (40 days) and the diapause incidence a little higher (34.3%) than might be expected from experience of fixed short days.(Figure 41). However this result was not sufficiently different to suggest an increase in diapause intensity.

These experiments were repeated with nymphs kept under increasing and decreasing photoperiods, and the results were substantially the same. For example, nymphs experienced

/declining

declining photoperiods from the 30th June (17 hours 20 minutes) to the 16 th August (15 hours) before feeding. All were in absolute diapause at 15°C, but this was broken for all the nymphs at a higher post-feeding temperature, 18.5°C (and LD 18: 6). Diapause intensity was no greater than in autumn field nymphs or nymphs experiencing fixed long days.

Photoperiods changing by small daily increments are recognised only as short or long photoperiods depending upon the absolute length of the light phase. Diapause incidence is not higher under declining photoperiods, nor when long photoperiods are followed by short ones (small daily changes across the threshold). These conclusions are supported by the field results; diapause incidence is high in July and August, and lower in September. This is also in agreement with Campbell's data (Campbell 1948).

There is evidence that larvae of I. ricinus, in some areas of its distribution, are unwilling to feed when they hatch in late autumn. No difficulty was found in feeding larvae which experience photoperiods equivalent to autumn field conditions.

5. Does chilling the diapausing ticks influence development in the following instars?

In an earlier section (III 3) it was suggested that late embryos might respond to the daylength conditions. In the field, diapausing embryos and other instars of the autumn population experience winter conditions of low temperature and short days, and after emergence, long days. Such a sequence of events

/when experienced

when experienced by unfed ticks, averts diapause.

Eggs from autumn females were kept under outdoor conditions overwinter or at 2-5°C in darkness for 16 weeks. The eggs were recently laid and could not have been in an advanced stage of development. After chilling, the eggs were incubated at 15°C and an 18 hour photoperiod. The larvae were fed after 4 to 6 weeks and 8 to 10 weeks in these conditions. The overwintering eggs were brought in on the 23rd March.

None of the larvae entered the moult phase in less than 150 days at 10°C (LD 18:6). At a higher post-feeding temperature (15°C) the pre-moult phase was short, minimum 18-21 days, but the incidence of diapause was very variable. In two laboratory populations and one field population it was high (66.6 to 75.7%), and in one laboratory and one field population low (20.7 and 36.3%). Larvae which experienced 8to 10 weeks of long days invariably had a high incidence of diapause. In similar experiments, larvae emerging from chilled eggs were fed after 3 to 6 weeks at a short photoperiod (LD 6:18 at 15°C). Diapause incidence was high, 55.5% and 62.7%, and the pre-moult delayed, minimum 31 to 35 days.

The response of unfed larvae to long or short days was only slightly modified by their embryonic experience. The incidence of diapause in some of the youngest long day larvae was low. In larvae from unchilled autumn eggs it was not less than 63.3%. However, the absence of development at 10°C and the higher incidence in other populations showed that chilling of the eggs had much less influence on subsequent development than chilling

/of the larvae.

of the larvae.

Chilling or overwintering experience of engorged larvae had no effect on nymphal development. Diapausing autumn larvae were chilled for 16 weeks at 2-5°C or kept under natural conditions near the laboratory overwinter. The larvae, while still in the pre-moult, were incubated at 15°C and an 18 hour photoperiod, and the nymphs fed 6 to 8 weeks after emergence. The minimum pre-moult at 15°C was not less than 100 days, but again, absolute diapause was broken at 18.5°C.

6. The irreversible nature of diapause after a prolonged period of induction in long days.

In the laboratory, it was difficult to prevent diapause after 8 weeks exposure of unfed ticks to an 18 hour photoperiod. Nymphs were kept for 2 or 8 weeks at 15°C and this daylength before being chilled for 20 weeks at 2-5°C in the dark. Even after this prolonged period of chilling the 8 week nymphs had a high incidence of diapause, 74%. For the two week nymphs it was much lower, 37.3% (Figure 42). (Page 72).

Nymphs which were exposed for only 2 to 3 weeks were little different from short day nymphs when favourable conditions followed. Starting with the same batch of engorged spring larvae, the experimental procedure shown overleaf was followed.

Nymphs emerged at 15°C LD 18:6
and were kept for 3 weeks.

to 15°C LD 14:10
for 5 weeks

Nymphs emerged at 15°C LD 14:10
and were kept for 8 weeks.

to 2-5°C in darkness for 14 weeks.

Fed after 6 weeks at 15°C LD 18:6

Development at 15°C after feeding is summarised below.

Initial experience at long days	Diapause incidence 38.5%	minimum pre-moult 18 days
Initial experience only short days	31.2%	18 days

In the previous sections, ticks with several weeks experience of long days have been avoided, but younger individuals have on occasions been used.

7. Termination of diapause in engorged ticks.

Diapause in the field must be terminated by winter conditions for development to proceed in the following spring. Chilling diapausing ticks in the laboratory at 2-5°C or 10°C lowered diapause incidence. This was less effective for the larvae and nymphs than overwintering or when chilling was followed by long days in the unfed ticks.

Nymphs collected in Perthshire on the 27th August and

/22nd September

22nd September, 1966, were kept at 10°C for two weeks after feeding. They were then chilled at 2-5°C in darkness for 2, 4 or 5 months, followed by long days at 15°C. The number in diapause was lowered particularly after 4 months chilling but the death rate was high. (Table 15).

Table 15. Development at 15°C and an 18 hour photoperiod of engorged Perthshire nymphs chilled at 2-5°C in the dark.

Nymphs collected 27th August.

DAYS AFTER CHILLING	TOTAL IN MOULT PHASE	TOTAL DEAD	DURATION OF CHILLING
20	0	1	2 months
30	0	4	21 nymphs
40	0	7	
50	0	9	
60	0	12	
110	0	20	
40	1	4	
50	4	8	
60	8	8	4 months
70	10	8	20 nymphs
80	11	8	
110	11	8	
20	0	2	
30	0	5	
40	0	9	5 months
50	0	9	20 nymphs
60	1	11	
110	2	18	
110	0	2	Unchilled control 27 nymphs

Nymphs collected 22nd September

20	0	0	
30	11	0	
40	13	2	4 months
50	15	2	23 nymphs
60	17	6	
60	0	0	Unchilled control 20 nymphs.

Diapause was terminated by chilling for some of the nymphs but the duration of the pre-moult was still prolonged compared with Perthshire nymphs. Of the 22nd September nymphs chilled for 4 months, 56.5% were in moult phase after 40 days. By this day, 95.5% of the 1st May population were in the moult-phase.

Development after chilling is still under photoperiodic control. In the experiments recorded in Table 15, equal numbers of nymphs were kept at 15°C and short photoperiod (LD 6:18), and all remained in absolute diapause.

The high death rate and poor results from these experiments suggest that the chilling has been experienced too soon, that is, before diapause has actually been established. The same nymphs collected on the 22nd September were kept at a higher temperature (15°C) and a short photoperiod (LD 14:10) for one month before chilling. After 4½ months at 2-5°C in the dark, 30 nymphs were brought out to 10°C and an 18 hour photoperiod. The first nymph in the moult phase was noted after 90 days and 72.4% eventually reached this stage. This was an improvement when compared with unchilled autumn nymphs, but development was slow and percentage in diapause high compared with early spring nymphs.

In a similar experiment, larvae derived from spring adults were exposed to an 18 hour photoperiod for 2 months before feeding. Diapause incidence at 15°C was high; all of the larvae at a short photoperiod (LD 6:18) were still in the pre-moult after one month. Some of them were then chilled for 4½ months before being put to 10°C and an 18 hour photoperiod.

/Development

Development was slow, and out of 40 larvae only 16 (40%) entered the moult phase in less than 200 days.

Under field conditions, overwintering autumn larvae and nymphs begin development earlier than spring fed ticks. The next instars emerge earlier and host seeking activity begins in autumn (Campbell 1948). The winter experience of these ticks has not been completely reproduced under laboratory conditions.

Chilling at 2-5°C was successful in terminating embryonic diapause, even when it was experienced by the engorged female. Twenty-nine females collected in Perthshire on the 28th June 1966, were put to 2-5°C in the dark the day after they were collected. After four months chilling, development was followed at 15°C and a long photoperiod (LD 18:6). Larvae began to emerge from 28 batches after 59 to 71 days, and in one batch after 120 days. This was comparable with spring populations, for example, in 11 batches from females collected on the 13th May, 1966, larvae began to emerge after 58 to 68 days and in one batch after 108 days. These results are in agreement with Campbell's conclusion, that the temperature relations of embryonic development are the same for spring, and for chilled autumn ticks.

Diapause in any population can of course be averted if engorged ticks are placed in favourable temperature and light conditions directly after feeding. Even when diapause has been established it can be broken in more favourable conditions. Autumn or late spring nymphs after several weeks in diapause at 15°C and an 18 hour photoperiod, soon enter the moult phase when moved to a higher temperature with the same light regime (18.5°C and LD 18:6). Spring nymphs in diapause at 15°C

/and a short

and a short photoperiod, soon develop under long days at the same temperature. Subsequent development depends to some extent on the duration of exposure to short days. Nymphs collected in April 1965 were fed and kept at a short photoperiod (LD 14:10 at 15°C) for 2, 8 and 12 weeks before being moved to a long photoperiod (LD 18:6 at 15°C). Thirty nymphs were kept permanently at the long and short photoperiod and 20 were moved up on each occasion. During the 105 days of the experiment, only 26.7% of the short day controls entered the moult phase; in the 8 and 12 week groups these were discarded before the shift to long days.

Diapause was not apparently determined after only 2 weeks in short days; their progress to the moult phase was delayed by less than a week (Figure 43). After 8 or 12 weeks of short days, subsequent development in long days was delayed, but all eventually entered the moult phase.

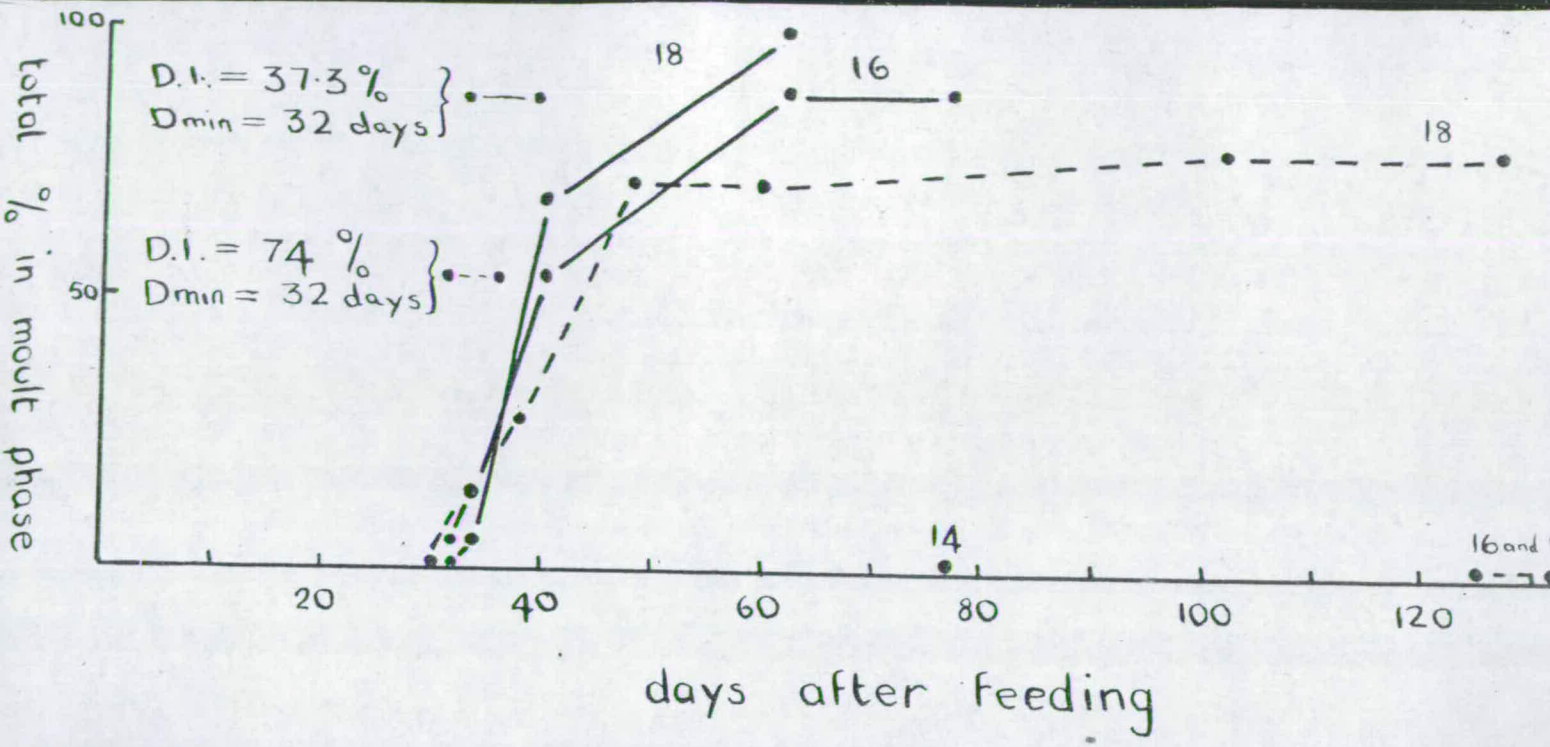
An interesting feature of spring populations is the synchrony in emergence of the next instars, even when ticks were fed early or late in the spring season (Campbell 1948). The short post-feeding photoperiods in early spring may contribute to this synchrony.

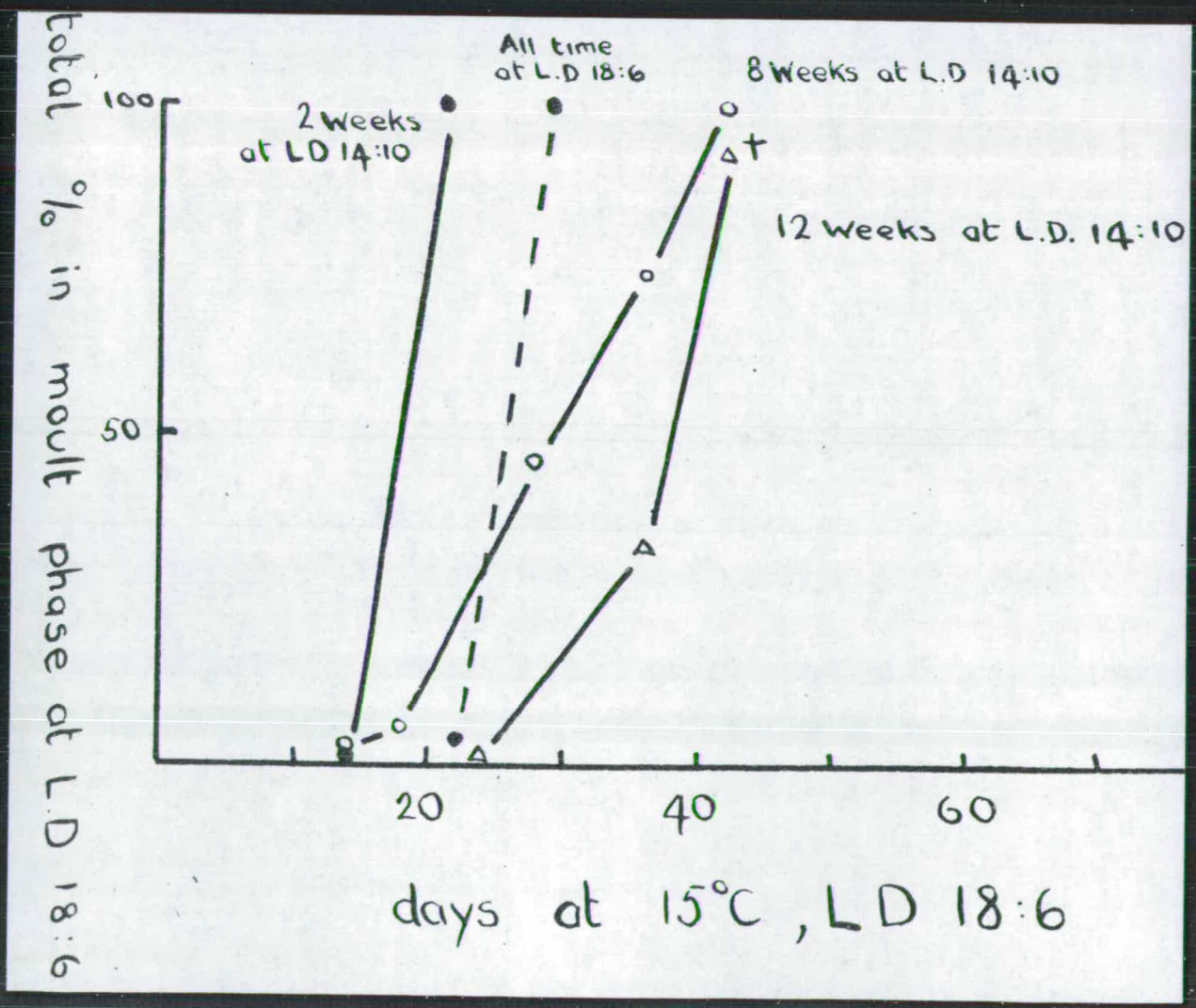
In the laboratory, when spring populations experience a photoperiod which is increasing by small daily increments, the early spring nymphs are delayed in the pre-moult until the threshold is reached. Nymphs collected in Peebleshire in March, April and May 1966, all entered the pre-moult at the same time when they experienced this photoperiod at 15°C. This

/was found

Figure 42. Development at 15°C of nymphs which experienced before feeding 2 or 8 weeks at 15°C and LD 18:6 followed by 20 weeks at 2-5°C in the dark. (broken line graph, 8 week nymphs).

Figure 43. Development at 15°C and an 18 hour photoperiod of nymphs collected in Peebleshire in April 1965.(broken line graph). Nymphs of the same population were fed and put to a short photoperiod (LD 14:10) for 2, 8 or 12 weeks before removing them to the 18 hour photoperiod.


total % in moult phase
100
50
D.I. = 37·3 %
Dmin = 32 days
D.I. = 74 %
Dmin = 32 days
18
16
18
14
16 and
20
40
60
80
100
120
days after feeding


total % in moult phase at L.D 18:6
100
50
All time at L.D 18:6
8 Weeks at L.D 14:10
2 weeks at LD 14:10
12 weeks at L.D. 14:10
20
40
60
days at 15°C, LD 18:6

was found also in 1967 for a Perthshire population of nymphs which experienced only natural conditions near the laboratory (Figure 44). It was also apparent from this experiment that engorged nymphs do not recognise small daily increases in the photoperiod.

Prolonged exposure of engorged ticks to short photoperiods at 15°C tends to delay development in subsequent long days, only in populations which already have a low intensity of diapause (spring nymphs). Where the intensity is already high (autumn nymphs) short days can to some extent replace chilling in lowering the intensity. None of the nymphs collected on the 27th August 1966 entered the moult phase in less than 200 days at 15°C and an 18 hour photoperiod. After 3 months at a 6 hour photoperiod (at 15°C), 25 nymphs of this population were moved to the longer photoperiod. Twenty-three (92%) entered the moult phase within 90 days of the switch. This was repeated on several occasions with autumn nymphs.

It is possible that, in the laboratory, some combination of short days and low temperatures might terminate diapause as effectively as natural winter conditions.

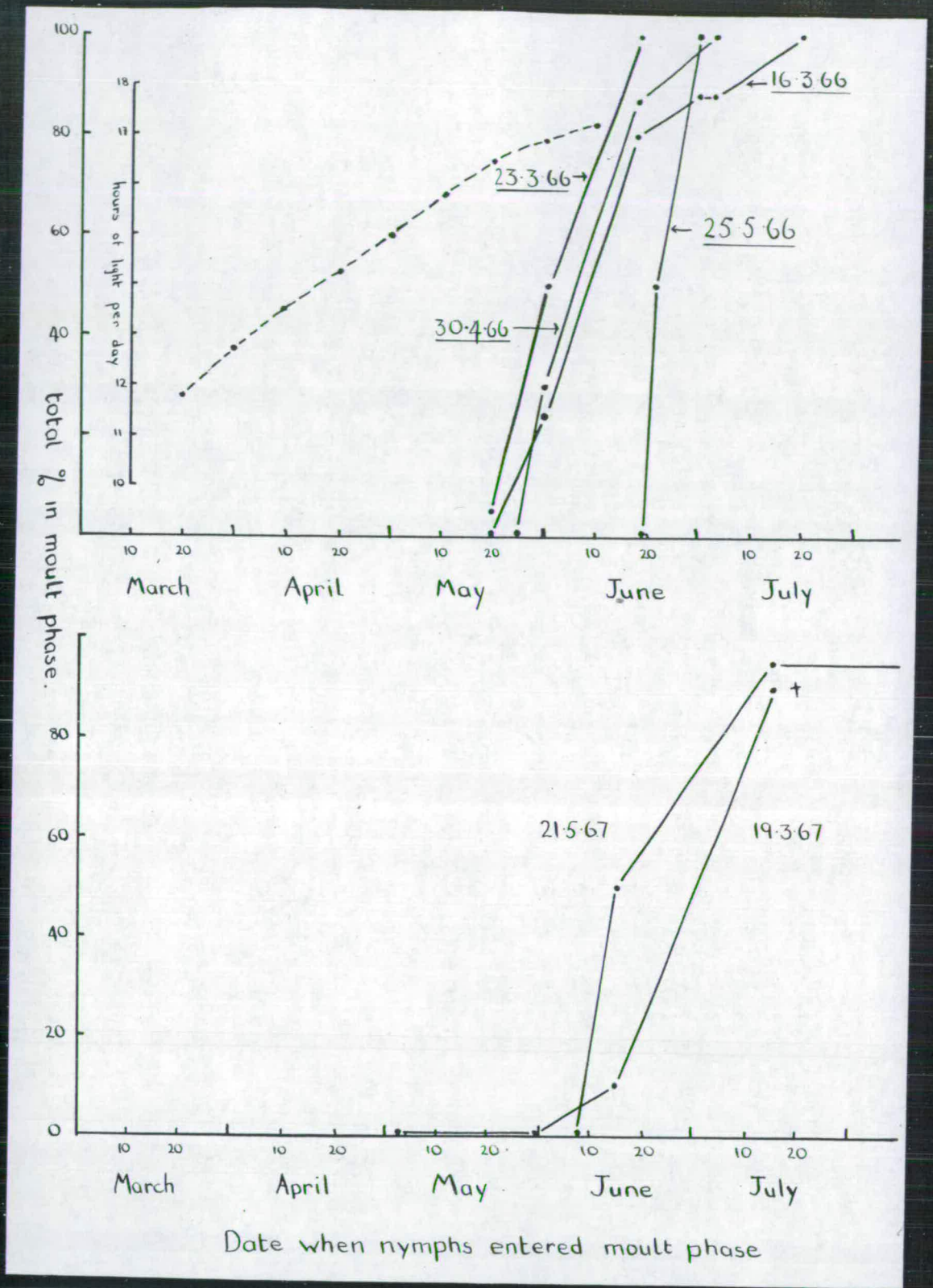


Figure 44. Development at 15°C and an increasing photoperiod of nymphs collected in the field in spring. Top graph; nymphs collected in Peebleshire 1966. Bottom graph; Development under the same conditions of nymphs which experienced outdoor conditions near the laboratory before feeding. The dates on the graphs indicate when the nymphs were collected.

V

PHOTOPERIODIC CONTROL OF DIAPAUSE IN OTHER IXODID TICKS

Diapause was found in several other Ixodid ticks. The life history of these species under natural conditions is, in most cases, not known in detail, and the significance of diapause could not be assessed. Only a few observations of interest are given for comparison with I. ricinus.

Females of I. hexagonus were sent from Cumberland, and their progeny were kept under bushes in outdoor conditions or were kept in the laboratory. I. canisuga in all stages of development were found in the stone walls of dog kennels and engorged adults were collected off the dogs. They were kept in a shed or in controlled conditions in the laboratory.

Larvae and nymphs of I. hexagonus, which experienced only outdoor conditions, were fed between the 9th and 11th September 1966, and again in spring, 22nd May 1967 (Figure 45). The autumn and spring populations were derived from the same population of ticks. In similar experiments with I. canisuga, larvae and nymphs were fed in autumn (12th November 1966) and again in spring (23rd March 1967) (Figure 46).

The intensity of diapause in both species was lower than for I. ricinus. Both pre-feeding winter experience and the post-feeding photoperiod at 15°C influenced development. As in I. ricinus, diapause was more strongly manifest in autumn and at short photoperiods.

Diapause could not be induced by conditioning the unfed ticks at any combination of temperature or photoperiod in the

/laboratory

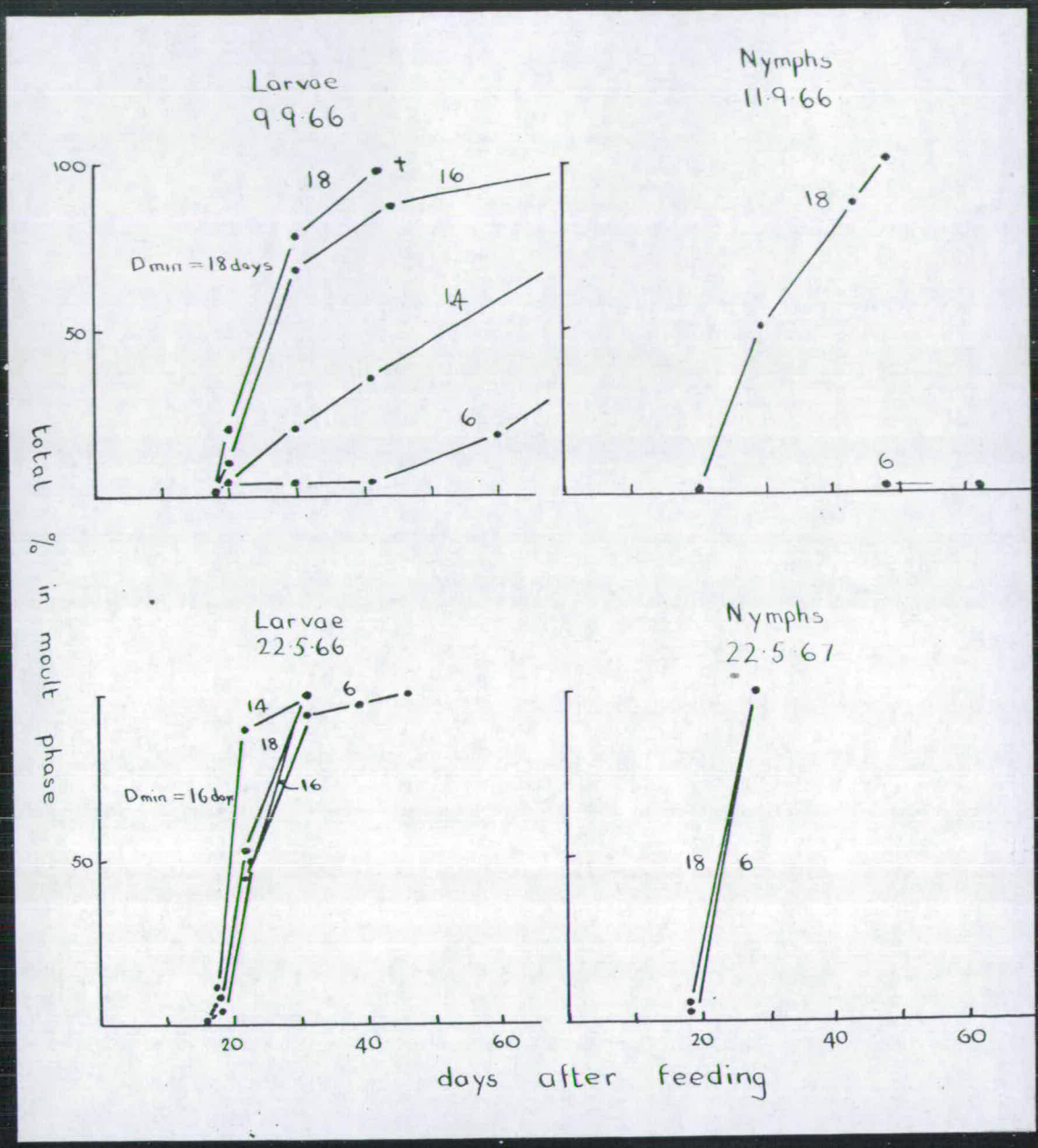


Figure 45. Development of I. hexagonus larvae and nymphs at 15°C. The unfed ticks experienced outdoor conditions only. Part of the population were fed in autumn (9-11th September) and the rest in spring (22nd May).

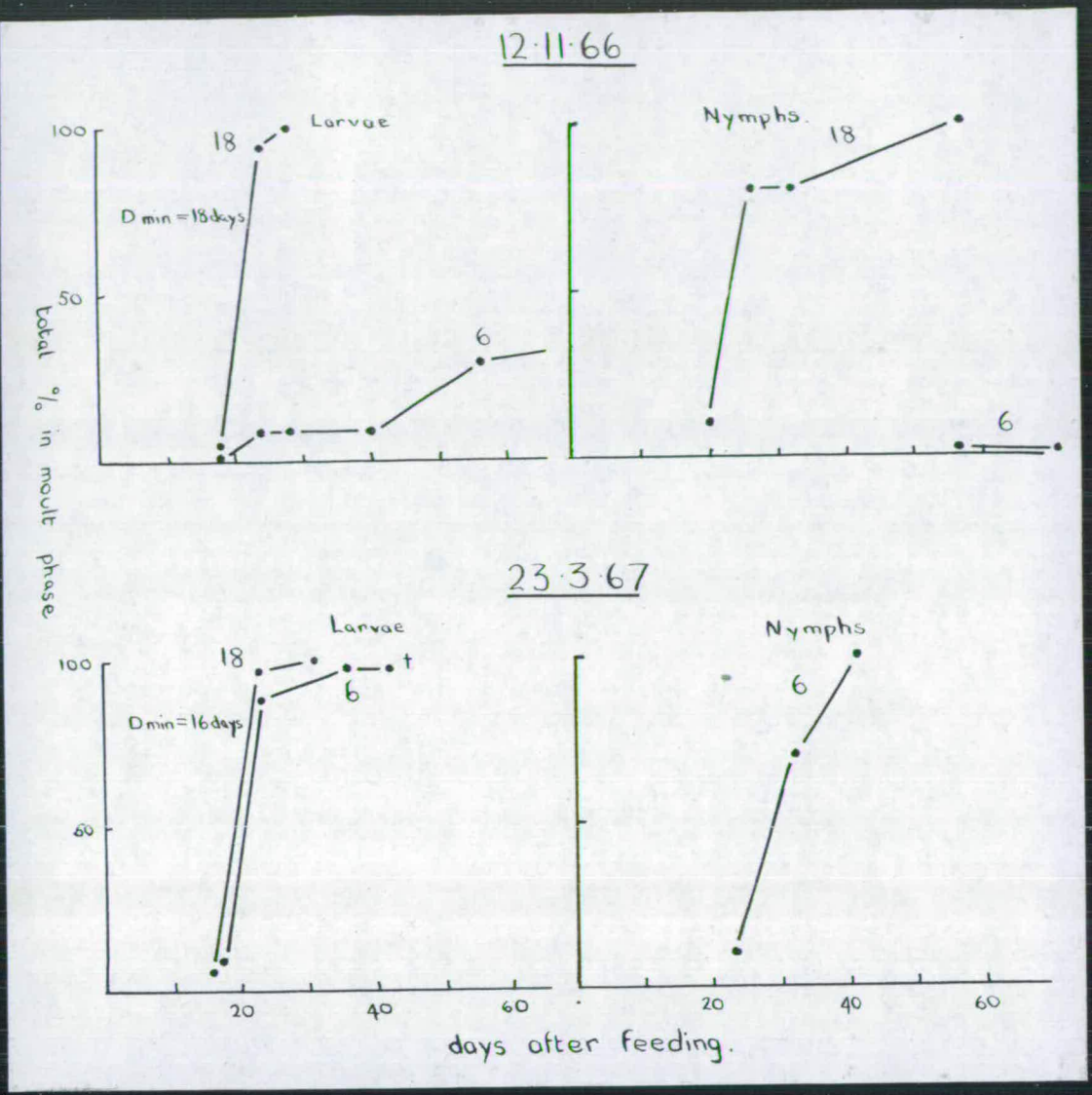


Figure 46. Development of I. canisuga larvae and nymphs at 15°C. The unfed ticks experienced outdoor conditions only. Part of the population were fed in autumn (12th November) and the rest in spring (23rd March).

laboratory. In situations where an intense diapause was induced in I. ricinus, only a short and inconsistent delay was found. Long photoperiods followed by short ones were however most successful and it is possible that the sequence of temperatures and photoperiods experienced in autumn is important.

A rather degenerate form of diapause is not unexpected in I. canisuga. It is well protected in its habitat from adverse conditions and has a ready supply of hosts. In I. hexagonus it is rather surprising, as this tick is found in more exposed situations and is associated frequently with a hibernating host, the hedgehog. The habits of this tick, off the host, are not well known.

Larvae of H. leporis-palustris have a higher intensity of diapause than the two Ixodes species. Adults, collected in June, were sent from Ontario, Canada. They were incubated at 15°C and a long (LD 18:6) or a short (LD 6:18) photoperiod. Larvae were fed after 6 and 16 weeks exposure. Diapause incidence was low in the younger long day ticks and high in the older ones (Figure47). This is similar to the response in larvae of I. ricinus, although the incidence is lower. Under short day conditions the response is again close to I. ricinus , diapause incidence being lower in the older ticks. The minimum pre-moult, after a long photoperiod, was only 14 days and after a short photoperiod it was prolonged to 28 days. There was an even greater tendency in H. leporis-palustris for a long pre-feeding photoperiod to assume a dominant role. Diapause was averted or induced irrespective of the post-feeding

/daylength

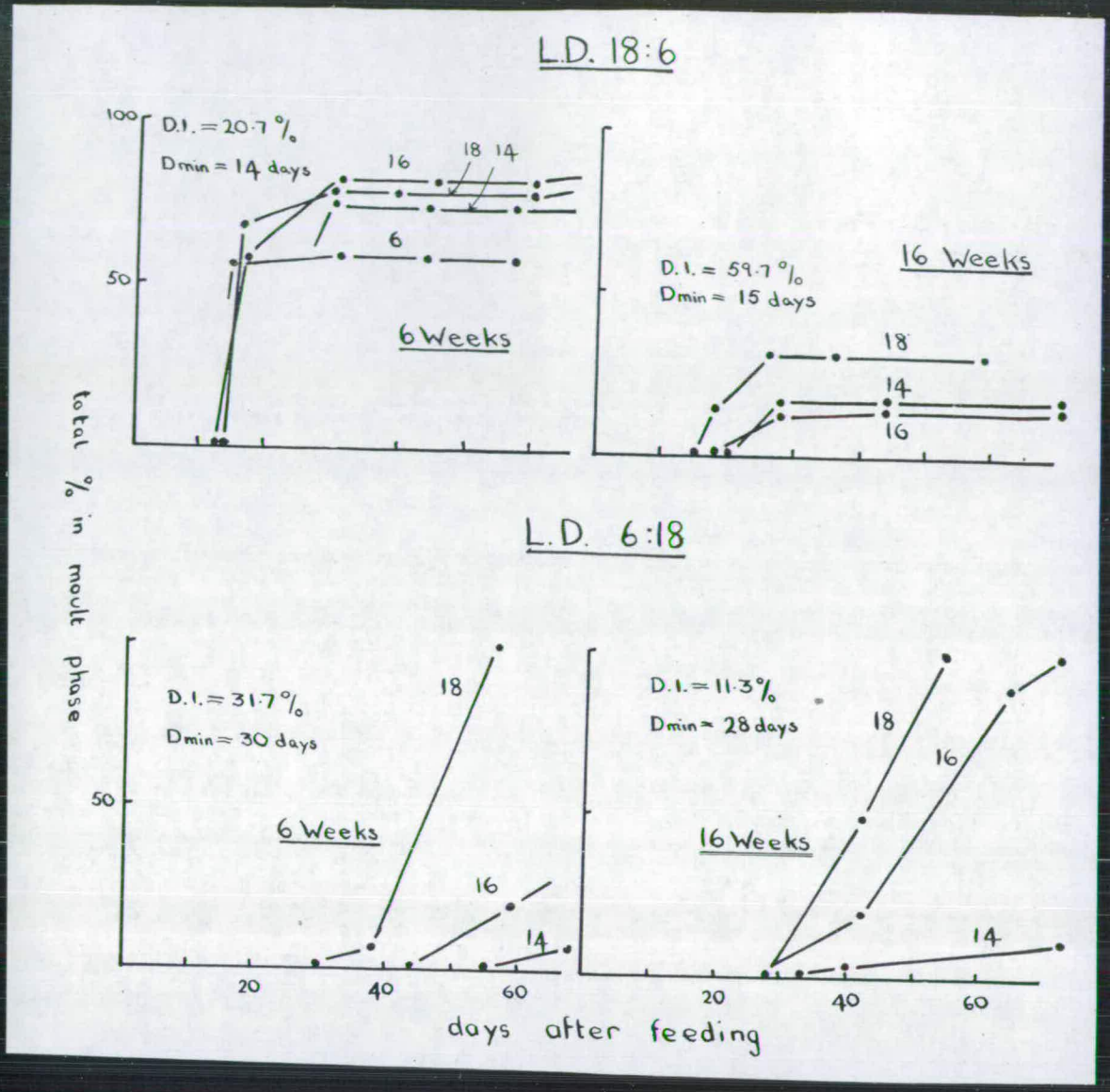


Figure 47. Development of H. leporis-palustris larvae at 15°C. The unfed ticks experienced 6 or 16 weeks at an 18 hour photoperiod (upper graphs) or a 6 hour photoperiod (lower graphs).

daylength.

A delay in the pre-moult phase was also found in larvae and nymphs of H. punctata. The original larvae were collected by blanket dragging on the Old Manor marshes, opposite Mersea Island, Essex. Nymphs which experienced 4 or 20 weeks at 15°C and a long photoperiod (LD 18:6) were all in diapause at 15°C after feeding. An increase from 4 to 20 weeks exposure to short days (LD 6:18) lowered diapause incidence from 83.3% to 43.3%. The response is again similar to I. ricinus. The post-feeding threshold was between 16 and 14 hours of light per day.

In this species, chilling unfed nymphs at 10°C was more effective than at 2-5°C in averting diapause. The incidence of diapause at 15°C, after chilling for 3 months at the higher temperature, was 34%, and 53.4% after chilling at the lower.

Adults of H. punctata did not feed well on rabbits. A large batch of males and females, derived from chilled flat nymphs, were put on the ears; only 8 females engorged fully and detached. No eggs were laid at 15°C and a long photoperiod (LD 18:6). In a second batch only 4 fully engorged females were obtained. No eggs were laid at 23°C and an 18 hour photoperiod although the females were still mobile after 3 to 4 months.

During feeding, males were found with their ventral surface closely applied to these females. It seems likely that copulation took place on the host, and that inability to lay eggs was not due to infertility of the females. Diapause in adults of H. punctata requires confirmation.

/Photoperiodic

Photoperiodic control of diapause seems likely to be a common feature of development in temperate species of Ixodid species. The photoperiod both before and after feeding may be important and, in the unfed ticks, the response to photoperiod may change with increased exposure.

Several tropical Ixodid ticks were reared in the laboratory but their development was not influenced by long or short days.

One experiment, with the Spotted Fever tick, D. andersoni, suggested that the inability to feed in midsummer and autumn may be associated with the daylength conditions. The ticks were sent from British Columbia, and the adults were chilled for 5 months at 10°C in the dark before feeding. The progeny of one female were reared to the adult stage at 23°C and an 18 hour photoperiod. The adults of this generation were either fed one month after emergence or were moved to other conditions before feeding. These conditions were 14 weeks at 15°C and three different light regimes, LD 16:8, LD 6:18, and a photoperiod declining from 17 hours 25 minutes to 10 hours 20 minutes. Farther batches were chilled for 16 weeks at 2-5°C in the dark, and 10°C at an 18 hour photoperiod or in darkness.

Normally feeding takes less than 10 days, and if any adults had not detached by this time they were pulled off. In each group 10 females and 10 males were used, and these were weighed after dropping or when removed from the host. The pre-feeding experience and the weights are summarised in Table 16.

Table 16. Pre-feeding experience and engorged weights of D. andersoni females (in grams).

Fed directly	4 weeks at 23°C, LD 18:6 → 15°C, LD 16:8 14 weeks	4 weeks at 23°C, LD 18:6 → 15°C, LD 6:18 14 weeks	4 weeks at 23°C, LD 18:6 → 15°C, declining 17 hrs 25 mins to 10 hrs 20 mins 14 weeks
0.81	0.42	0.52	0.42
0.71	0.38	0.47	0.05
0.69	0.37	0.37	0.05
0.66	0.31	0.36	0.04
0.65	0.31	0.30	0.02
0.62	0.29	0.23	0.01
0.60	0.15	0.23	0.01
0.58	0.03	0.20	0.01
0.58	0.01	0.15	0.01
0.10	0.01	0.10	1 died.
mean 0.60	mean 0.23	mean 0.29	mean 0.06

4 weeks at 23°C LD 18:6 → 10°C, LD 18:6 16 weeks	4 weeks at 23°C LD 18:6 → 10°C, darkness 16 weeks	4 weeks at 23°C LD 18:6 → 2-5°C, darkness 16 weeks
0.82	0.67	0.74
0.73	0.65	0.66
0.69	0.63	0.66
0.67	0.63	0.63
0.62	0.61	0.58
0.57	0.61	0.55
0.50	0.59	0.48
0.49	0.57	0.47
0.43	0.57	0.44
0.40	0.33	0.36
mean 0.59	mean 0.59	mean 0.56

After a prolonged experience of moderate temperatures (15°C), the adults become less willing to feed. This is not found at lower temperatures (10°C or 2-5°C). The ticks at a declining photoperiod have a more recent experience of long followed by short days and this may account for their very low weights after 10 days on the host. The rabbits in these experiments were all being used for only the first or second

/time

time. No form of host resistance was observed which could explain the results.

These possibilities clearly require farther investigation, particularly in relation to the host seeking behaviour of this tick.

VI MIDSUMMER INACTIVITY OF IXODES RICINUS

Activity was not observed in the tick habitat. Adults and nymphs were confined in large glass jars 18 cms. high and 10 cms. in diameter. The tops of the jars (8 cms. in diameter) were closed with one layer of thin muslin, mesh size approximately 0.1 mm. Soil, leaves and moss formed a basal layer 3 cms. in depth, and from this several grass stems were growing. During dry weather the vegetation was kept moist. The jars were placed under bushes near the laboratory and these protected them from direct sunlight and excessive moisture.

Larvae and nymphs, for these experiments, were collected in Peebleshire on the 5th and 26th May 1966. They were fed in the laboratory and returned to the field. The new instars began to emerge in August and on the 8th October they were transferred to the activity jars near the laboratory. They experienced at all times only natural light conditions.

Activity was followed from October 1966 to September 1967. Ticks were active in October and 5 females were removed and fed successfully. There remained in the activity jars 53 nymphs, 43 females and 25 males. By the end of February 10 nymphs, 12 females and 8 males were found dead in the basal layer, but this was not thoroughly examined and some dead ticks may have been missed. The population could not have been more than 43 nymphs, 31 females and 17 males.

Ticks were considered active if they were seen above the basal layer, either on the stems, on the sides of the jar or on the muslin/

muslin top. To count these ticks the jars were gently removed from the bushes and placed on a large stone still in the shade. During April and May this movement enticed more ticks from the basal layer. Gently blowing into the jars or warming in direct sunlight had the same effect. Counting of active ticks was started from the basal layer upwards and counting of these additional ticks was strictly avoided. The results recorded in figure 48 include, therefore, only spontaneously active individuals.

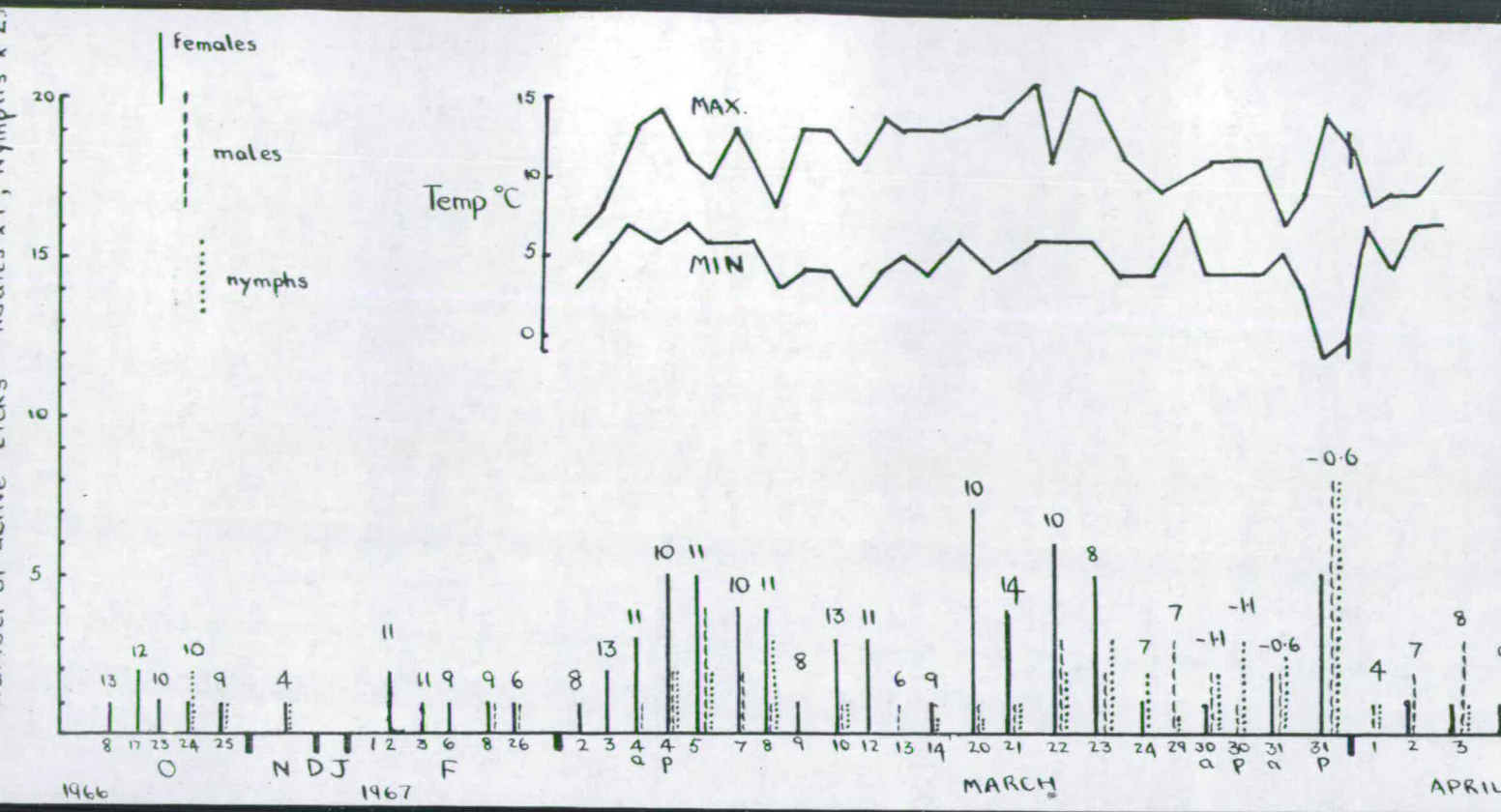
During the time of greatest spontaneous activity (March to May) the jars were occasionally opened and all the active ticks knocked down to the base. This was performed, on average, once a week during these months. Admittedly the ticks were considerably disturbed during their whole period in the jars, but this is not an unnatural situation. If the disturbance was greater than normally experienced, then the ticks would be expected to exhaust their reserved of food and die earlier than in the field. On the contrary, these individuals survived longer than has been recorded for field ticks.

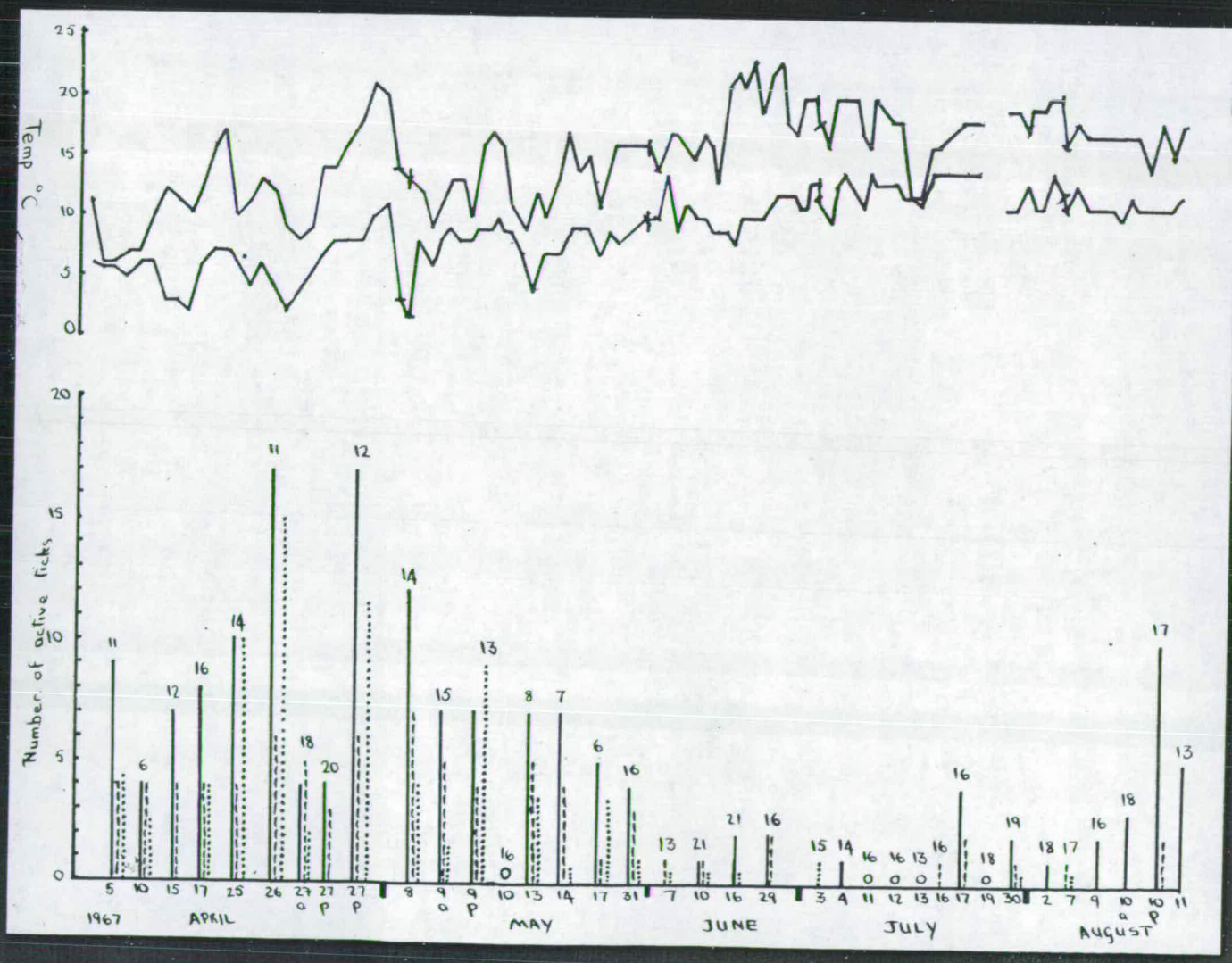
The jars were observed at irregular intervals, at different times of the day and, on occasions, up to three times in a day. The time of day is recorded only as A for the morning, and P for the afternoon (Figure 48). The temperature close to the jars was recorded at the time of counting and the maximum and minimum temperatures for the year were also taken. The information is again summarised in figure 48.

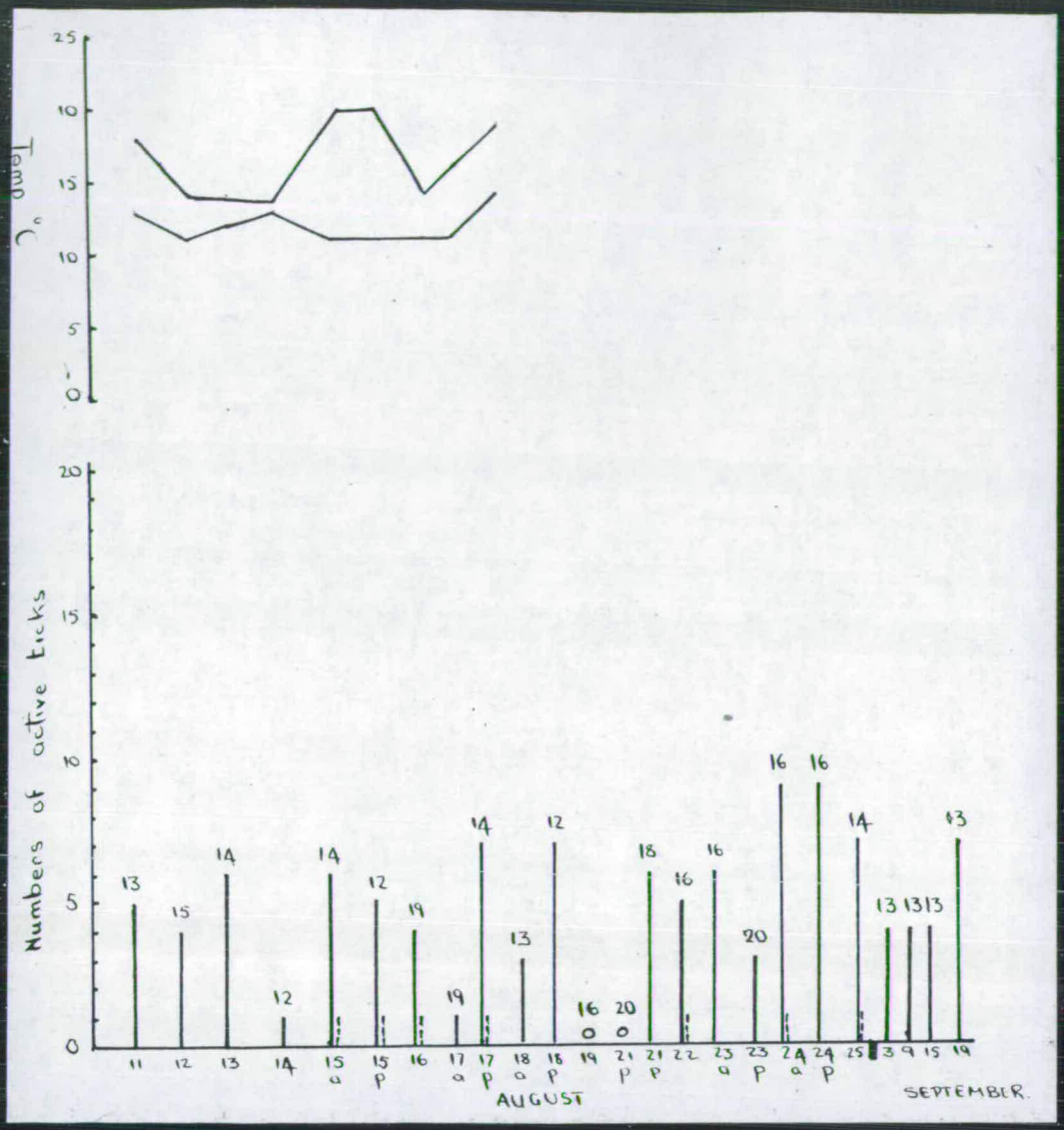
Activity, of a few individuals of these spring populations, was found in late autumn, a few weeks after the new instars had emerged./

Figure 48. Activity of I. ricinus adults and nymphs in outdoor conditions near the laboratory. The temperature in °C at the time of activity counts is recorded above each column. The date is recorded at the foot ofthe column.
a. counted during the morning.
p. counted during the afternoon.

Continued over-leaf.


females
males
nymphs
Temp °C
MAX.
MIN
Number of active ticks
1966
1967
O
N
D
J
F
MARCH
APRIL


Temp °C
Number of active ticks
1967
APRIL
MAY
JUNE
JULY
AUGUST


25
20
15
10
5
0
Temp °C
Numbers of active ticks
20
15
10
5
0
13
15
14
12
14
12
19
19
14
13
12
16
20
18
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23 a
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25
3
9
15
19
AUGUST
SEPTEMBER.

emerged. On any occasion this was not more than 4.2% of the females and 7.5% of the nymphs. Activity ceased during winter, a few adults were seen in February, and by March ticks were active at every count. During April and early May activity reached a peak, but the numbers declined towards the end of May. In June, July and the first few days of August few ticks were seen. On the 10th August, 10 females had emerged from the basal layer. From then, until the experiment was terminated on the 19th September, consistently higher numbers of females were active than in the midsummer period.

Death of the nymphs may have contributed largely to the late spring decline in their activity. This was not true for the adults, as many reappeared in autumn. During midsummer they could not be enticed into activity by moving the jar, banging it on the ground or warming it in direct sunlight for several hours. On ten occasions during the summer no farther activity was found after any of these treatments.

On the same ten occasions gently blowing into the jars had a marked effect. As many as 13 females, 3 males and 5 nymphs emerged from the basal layer and began to climb rapidly up the stems. (Figure 49). The ticks were disturbed in this way for the last time on the 7th August. By the 9th all but two females had again disappeared into the vegetation layer. The recurrence of spontaneous activity on the 10th probably did not result from their disturbance at an earlier date.

Mammalian Breath must be recognised by the inactive ticks. They may be sensitive to higher concentrations of carbon dioxide, and this is the only possibility which has been tested. On the afternoon/

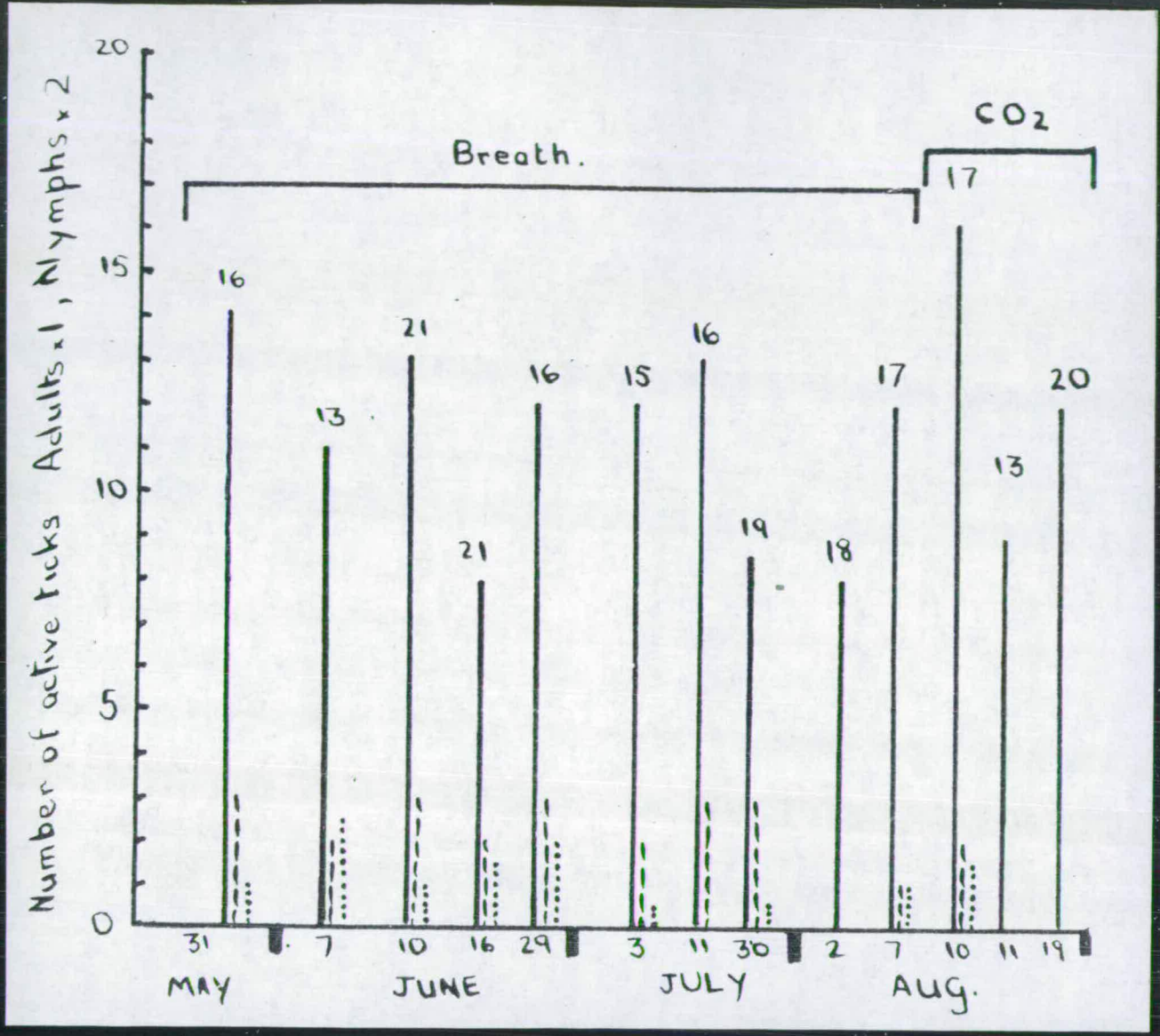


Figure 49. Activity in the same population as shown in figure 48. Breath and CO_2 induced activity during the midsummer inactivity period.

afternoon of the 10th August, not all the available ticks had resumed activity. Moving the jar had no farther effect, but a few small crystals of dry ice placed on the muslin top induced 6 more females and 2 nymphs to emerge and climb to the top of the jar. On the 11th August, carbon dioxide induced 3 more females to begin activity. On the morning of the 19th August, no females were observed 5 minutes after moving the jar. Two minutes after applying the carbon dioxide a total of 13 females had emerged.

Carbon dioxide had a striking effect on the activity of ticks which were already on the stems, but which had assumed the characteristic 'resting posture' observed by Lees (1948). They immediately unfolded the first pair of legs and, after a brief period of questing, invariably began to climb up the stems. Noxious materials might also ellicit this response, but changes in the environment which are associated with the proximity of the host are likely to have greater biological significance. The climbing response observed when very small quantities of dry ice were placed on the muslin, did not suggest that the ticks were taking an avoiding reaction. Carbon dioxide is known to attract three tick species, O.coriaceus Koch, 1844; D. occidentalis Marx, 1892 and I. pacificus Cooley and Khols, 1943. (Garcia 1962).

These experiments were repeated, with similar results, using a smaller Perthshire population (32 adults).

The cessation of spring activity was not caused by death of the hungry adults. Nor does it appear to be related to adverse conditions. Activity was resumed in August at higher temperatures, and the contents/

contents of the jar were kept moist in summer. Long photoperiods alone were probably not responsible. Activity was continued into May, but at equivalent summer photoperiods no ticks were found.

Developmental diapause is associated with the pre-feeding experience of early June nymphs. Experiments under controlled conditions suggest that this diapause is induced by the persistance of long days at temperatures above 10°C. Midsummer inactivity may be a rather unusual form of diapause, under the direct control of the temperature and daylength experience. This is a possibility mentioned by Belozerov (1964a) concerning midsummer inactivity in D.marginatus but he has laid greater emphasis on the photoperiodic conditions. Another possible explanation arises from the recent observations by Atwood and Sonenshine (1967), on the activity of D.variabilis. Field temperatures were not important in controlling activity. There was a direct relationship between the number of active larvae and adults, and a level of solar radiation of 300 or more langleys/day. An inverse relationship, if it applied to I.ricinus, would prevent activity in summer.

In I.ricinus the problem of midsummer inactivity is likely to be of interest rather than practical importance. In Peebleshire, on the same farm from which the experimental ticks were taken, no recrudescence of activity occurs in autumn. Campbell (1948), in a thorough examination of the vegetation mat, found no viable ticks on this farm in summer. In the tick habitat in Northumberland, little 'spring activity' was found after July even when the main host (sheep) was excluded from the area. (Lees and Milne 1951). Although observations were continued into autumn the number of active ticks/

ticks remained very low.

The results recorded here suggest that I.ricinus females have a greater capacity for survival than expected from other activity experiments (Lees 1964).

VII THE LIFE HISTORY OF *Ixodes ricinus* UNDER NATURAL CONDITIONS.

Although comparison of events in the field and under controlled conditions in the laboratory is a procedure which must be applied with caution, the laboratory data presented here are readily reconcilable with the field evidence. It is possible to assess, to some extent, the contributions made by the temperature and daylength in regulating the life cycle.

Spring ticks have all experienced short photoperiods and low temperatures in the unfed state in autumn and over winter. Those which feed early, in March and April, have experienced only low temperatures and short days. Those which feed later in May have an additional experience of long days and higher temperatures, and this difference is reflected in the lower incidence of diapause and shorter premoult with the progress of the season.

Diapause becomes increasingly manifest with the persistance of higher temperatures and long days. In Scotland, when feeding is naturally delayed until June some of the nymphs develop rapidly and some diapause. After experimental delay in the field until July diapause is strongly manifest in all the nymphs. When larvae were delayed until the beginning of July an increase in diapause incidence was revealed only at low post-feeding temperatures. By the end of July however diapause was found even at higher temperatures.

By the end of June there was a delay in the preoviposition period of engorged females, and in the embryonic development of their progeny, but there is little direct evidence to associate this/

this with persistant long days and higher temperatures experienced by the hungry adults.

In the field, delay in development of early spring fed ticks is of course not entirely due to their pre-feeding experience. Both Campbell (1948) and Babenko (1956) found synchronous emergence of spring ticks which they attributed to the low temperatures experienced by the early fed ticks. For example in 1944 Campbell observed adult emergence from March and April fed nymphs between the 28th August and the 9th October. Emergence from May and June fed nymphs was in fact slightly earlier, between the 14th August and 25th September.

An attempt was made to determine the relative importance of low temperatures or short days in delaying spring fed ticks. Nymphs collected in March and April 1966 were fed, and kept in tubes in outdoor conditions near the laboratory. A Philips 8 watt fluorescent bulb was suspended 1 foot above the tubes and the daylength extended each evening to not less than 16 hours of light per day. Other tubes experienced only natural conditions.

Both the experimental and control groups entered the moult phase at the same time, between the 20th June and 15th July. Unfortunately this result can not be taken as conclusive evidence that low temperatures alone are delaying early engorged ticks. The extended light period was experienced during the evening when the temperature had fallen to between 5° and 10°C. It is possible that at these temperatures the additional light was not recognised by the internal 'clock'. Such a situation has been envisaged by Danilevskii from experiments with Acronycta rumicis. (Danilevskii/

Danilevskii 1965, page 124).

Low temperatures and short days both before and after feeding tend to operate conjointly to delay early spring ticks. Engorged ticks pass readily from diapause to development as the temperature and photoperiod increase. Later spring ticks develop more rapidly and synchrony is maintained in the whole population.

Diapause is not usually associated with spring ticks. In the Moscow region the first diapausing nymphs were not found until the end of July (Babenko 1956), and Campbell (1948) attributed diapause in the July fed ticks to the high temperature experience of the newly emerged autumn population. It is interesting that farther north in Karelia, where the active ticks are derived only from overwintering flat ticks, diapause is found in up to 60% of the June fed ticks depending upon the particular season (Kheisin et.al. 1954a). In Scotland diapause in late spring ticks was found in the laboratory and also in the field. Perthshire nymphs fed on the 1st May, 28th May and 12th June 1966 were returned to the field. Of 131 1st May nymphs, 124 had moulted to adults and 6 had died by the 22nd September of the same year. By this date 37 out of 40 nymphs fed on the 28th May had moulted, one was still in the pre-moult and 2 had died. Diapause was however strongly manifest in the 12th June ticks, of which 43 out of 50 were still in the pre-moult, 4 had died and only 3 had emerged as adults. The diapausing individuals overwintered and did not moult until the following summer.

Spring nymphs which were experimentally delayed in the field until feeding on the 19th July, all entered diapause. The sample here was small (21 nymphs), but in 1967 the experiment was repeated/

repeated with 66 nymphs delayed until the 30th July, and again all were in diapause in the field.

In similar experiments with spring field larvae, large numbers were returned to the field from populations fed up to the 3rd July 1966. Not less than 98% of all these larvae had time to moult in the same season, but of 60 delayed in feeding until the 31st July not one entered the moult phase until the following year. It is worth recording that in egg batches derived from 10 Perthshire females collected and put to the field on the 12th May 1966, very few unhatched eggs were found by October of that year. In egg batches laid in July from 10 females collected on the 28th June, no larvae emerged until the following year.

It has already been argued that these females were probably delayed spring ticks. Diapause in experimentally delayed spring larvae and nymphs has been shown but the nymphs collected in June might represent the first of the autumn populations. This seems most unlikely. In 1966 and 1967 the first nymphs did not emerge from overwintering engorged larvae until July, and in Campbell's observations autumn nymphs did not emerge earlier than mid-June and activity did not begin until July (Campbell 1948). June nymphs are presumably the last of the spring active Ticks.

Diapause in June and July, when post-feeding temperatures and photoperiods are most favourable for development, must be attributed to the pre-feeding experience of these spring ticks. Persistance of higher temperatures and long days could account for this phenomenon.

The intense diapause in all the early autumn nymphs is readily/

readily reconcilable with the laboratory observations. Nymphs emerging in long days, from chilled or unchilled engorged larvae, all diapause under favourable conditions for development. The lower incidence of diapause in late autumn can be related to the longer pre-feeding history which includes both low temperatures and short days.

The relative importance of pre-and post-feeding conditions in ensuring universal diapause in autumn is a matter for conjecture. Clearly there is a tendency for pre-feeding conditions to assume the dominant role in early autumn, and post-feeding conditions in late autumn. The photoperiod probably does not influence embryonic diapause but for the larvae which have the lowest intensity of autumn diapause it may have greater significance.

The progression of larval development in autumn is slightly different from the nymphs. Among the earliest larvae fed on the 2nd August 1966 a small proportion developed rapidly at 15°C and in the field 12 out of 63 had moulted by the beginning of October. Later larvae returned to the field were all in diapause. It can be shown in the laboratory that after a short experience of an 18 hour photoperiod, larval diapause intensity is still low and that development can proceed rapidly at 15°C and above. In a few of the earliest autumn larvae the experience of long days may be sufficiently brief to induce rapid development rather than diapause. For the majority however the experience was sufficiently prolonged to favour diapause.

Autumn ticks may not find a host until the following spring and in spring ticks the whole population overwinters. Prolonged exposure/

exposure to low temperatures and short days is a natural experience of these ticks and both may contribute to the lower incidence of diapause found in the larvae and nymphs in early spring. Embryonic development may be influenced in a similar way by the overwintering experience of the unfed adult but this requires experimental confirmation.

Diapause, once established in the autumn ticks, is broken during the winter and development is resumed in favourable spring conditions (Campbell 1948). In the laboratory, chilling at 2-5°C in the dark followed by a higher temperature and long photoperiod, was sufficient to terminate embryonic diapause. Even incubation at 10°C at a long or short photoperiod had a marked effect. These conditions were only partly successful in terminating diapause in larvae and nymphs. It is difficult to envisage a more favourable sequence of events, but it is possible that diapause in these stages has a lower temperature requirement, which has been satisfied only under field conditions.

The regulation of diapause and development in autumn and spring populations of I.ricinus is presumably important in the adaptation of the life cycle to the unstable environment. Midsummer inactivity of unfed adults may also contribute to this end. It does not appear to be related to adverse conditions at this time of year.

This interpretation of the life history of I.ricinus in Scotland is not in agreement with the conclusions of Belozerov concerning Russian populations. In his first experiments with engorged larvae of a Leningrad population, the threshold photoperiod at 18°C lay between 17 to 18 hours per day. A higher temperature, 25°C/

25°C, tended to avert diapause even at short photoperiods. He concluded that in the field, long midsummer days were most important for rapid development, and that diapause which was found in late July was brought about by short days. This response to photoperiod was compared with that of long day insects.

In these experiments the pre-feeding photoperiod was not explicitly stated, but hungry larvae were kept at a range of temperatures, one group at 15°, 20° and 25°C and another at 5°, 15°, 18° and 20°C. In both groups diapause was lowest in larvae which experienced the highest temperatures 25° and 20°C, and highest in larvae which experienced 15° and 18°C. He made no comment on the relatively low incidence of diapause which was apparent in the larvae which were chilled at 5°C. He concluded that low pre-feeding temperatures favour diapause. This may not be a correct interpretation of the results, since the duration of exposure to the different pre-feeding temperatures was not the same. Larvae at the higher temperatures experienced only 1 or 2 months and at the lower 2 or 4 months.

The post-feeding temperature of 18°C in these experiments may be too high to make valid conclusions on field development. In one experiment where post-feeding temperatures of 15° and 18°C are compared, larvae had a high percentage in diapause at the lower temperature even in favourable light conditions. He quotes a mean midsummer maximum of 16°C in the tick locality.

Difficulty was also found in terminating diapause in engorged larvae of this population. Chilling at 5°C for 3½ months only delayed slightly subsequent development. His results, however, were/

were not sufficiently extensive to warrant the conclusion, that the photoperiodic response is unchanged by chilling or by the overwintering experience in the field (Belozerov 1964c).

Belozerov's experiments were repeated by Babenko and Platonova (1965) using larvae of I.ricinus collected in the Moscow district and a Far Eastern population of I.persulcatus. After chilling, the hungry larvae were however kept at a long or a short photoperiod for 30-40 days before feeding. Diapause incidence was lower after exposure to long days, showing for the first time photoperiodic sensitivity in hungry ticks of these two species. These authors made no comment on the influence of the initial period of chilling. Their results have been confirmed in similar experiments with a Scottish population of I.ricinus.

In more extensive experiments with the Leningrad population, Belozerov and Michailova (1966) kept hungry larvae for 2 months at long and short photoperiods. They confirmed that long days both before and after feeding were optimal for development. The long photoperiod before feeding was particularly important; the temperature during this time had little effect.

The incidence of diapause was extremely high after experience of short days. After two months at LD 12:12 (at 25° or 18°C), between 50-80% diapaused at a long photoperiod (LD 20:4) and a high temperature (18°C) after feeding. This ~~intensity~~ incidence of diapause was not found in any Scottish population of larvae derived from the field or the laboratory. There might be a true difference between the geographic races in the induction of intense diapause. However the/

the Leningrad material was kept, from the time of adult engorgement until the larvae were 5-10 days old, at 25°C and continuous light. They experienced a decline in photoperiod and in some cases also in temperature, before feeding. Although an increase in diapause intensity was not associated in a Scottish population with a decrease from long to short days, this was not exhaustively studied. It is possible that the discrepancy lies in the different experimental treatments.

Belozerov and Michailova believe that long day experience both before and after feeding leads to rapid development of ticks fed in early summer. The experiments recorded here suggest that for some early autumn populations in Scotland this may be true. It can be added that long photoperiods experienced by hungry larvae are much more effective after chilling, and that persistance of long days in chilled and unchilled larvae leads to diapause.

In the absence of chilling development in the field is not successful. Laboratory populations of larvae experienced 2 months at 15°C and LD 18:6 or this same experience preceeded by 5 months of chilling. Both groups were fed and put to the field on the 21st May, 1967. By the 15th September only one out of 101 unchilled larvae had moulted, while nymphs had emerged from 50 out of 55 chilled larvae and 5 had died.

Belozerov (1966) has recently published results from experiments on nymphs derived from I.ricinus females collected in Moldavia (47°N). Details of their laboratory history were not given, but the nymphs themselves experienced not less than 5 months of long or short photoperiods, and were again incubated at 18°C after feeding. The short day nymphs had the lowest incidence of diapause and he concludes/

concludes that nymphs, unlike larvae, have a short day requirement in the unfed stage. It must be noted that the larvae in his previous experiments were fed after only 2 months exposure. Experience with I.ricinus suggests that larvae after 5 months might approach the nymphal photoperiodic response, particularly if a range of post-feeding temperatures are used in the experiment.

It is possible that the conflicting data may become reconciled when the different populations are compared under a wide range of temperature and light conditions. This may reveal on the other hand alternative forms of diapause regulation in the widely separated populations of I.ricinus.

VIII PHOTOPERIODIC CONTROL OF DIAPAUSE IN IXODES RICINUS AND OTHER ARTHROPODS.

The majority of Arthopods in which photoperiod controls diapause can be considered as long day species. Long days and high temperatures favour development, while short days and low temperatures favour diapause. The daily light-dark cycles serve only as a token stimulus initiating the processes which lead to diapause at a later stage. Diapause is terminated at low temperatures and development resumed under favourable conditions.

In short day species, of which the best known is Bombyx mori, the response is reversed, diapause being induced after experience of long days and high temperatures. (Kogure 1933). Recent reviews by Andrewartha (1952), Lees (1955), Wilde (1962) and Danilevskii (1965) give examples of typical long and short day species and also other groups in which there is a greater plasticity in response to environmental stimuli.

This is particularly true of I.ricinus. The diapausing stages, except probably the egg, retain sensitivity to the temperature and photoperiod and respond in the same way as long day species. However chilling also has some effect in terminating diapause, and persistance of short days can raise or lower the intensity of diapause.

The unfed ticks are analagous to the sensitive stages in other Arthropods but are not typical of either long or short day species. The life cycle is so protracted that any stage in development can be exposed to favourable or unfavourable conditions. Under these circumstances each individual must be able to make a response/

response appropriate to the season in which it feeds and in this respect the most important adjustments are found in the hungry ticks. Thus, persistence of long days and high temperatures indicate the approach of unfavourable conditions and an intense diapause is induced. Persistence of short days and low temperatures immediately followed by long days and higher temperatures indicates favourable conditions and development subsequently proceeds rapidly.

This multiplicity of response ensures that whenever an individual feeds, development proceeds in harmony with the environment. Perhaps the most interesting parallel among insects is found in the tropical locust Nomadacris septemfasciata, where the sequence of photoperiods synchronises development of the younger stages with the wet season. Egg laying begins without delay only when the immature instars experience short days and the adults long days. The diapause associated with the direct action of short days on the adults can be intensified if the hoppers experienced long days. Photoperiods declining by small daily increments were no more effective than one step down from above to below the critical photoperiod. (Norris 1965). The bollworm Heliothis zea also has a different response to 'spring' and 'autumn' conditions. Diapause is manifest only when the preceding stages experience long followed by short days (Wells and Adkisson 1966).

Diapause in some insects is terminated in long days but the sequence of photoperiods may also be important. In the silkworm A. pernyi long days shorten the duration of pupal diapause. Brief exposure to short days (4 weeks at LD 8:16) delays emergence in subsequent long days, but prolonged exposure 17 to 22 weeks, even at a high temperature 25°C, had a similar effect to chilling. Diapause was terminated rapidly after transfer to long/

long days. (Williams and Adkisson 1964).

Plants also respond to the sequence of photoperiods and several 'short-long day' species are known. (Lang 1952). For example, in Winter Rye vernalization can be replaced to some extent by exposure to short days, the time to flowering being reduced after removal to long days. (Purvis 1934).

Diapause in several species, including I.ricinus, can be induced directly by short photoperiods and terminated by long ones. Larvae of Dendrolimus pini diapause under short day conditions, and development is readily resumed in long days. (Gayspitz 1949). Diapause in the fourth instar larvae of an autumn population of Aedes triseriatus can be induced and maintained in darkness, development is again resumed in continuous light. (Love and Welchel 1955).

Several other examples of this type of photoperiodic control were mentioned by Wilde (1962). Since then long days have been shown to terminate larval diapause in Pectinophora gossypiella (Wellso and Adkisson 1964), and pupal diapause in the two Saturniids Hyalophora cec cecropia and Antheraea polyphemus. (Mansingh and Smallman 1967). In other species in which photoperiod is assumed to have no effect, prolonged chilling of the diapausing stage may have obscured a photoperiodic response. Certainly in the spruce budworm Choristoneura fumiferana long days were more important for diapause termination in inadequately chilled larvae. (Harvey 1958).

The diversity of response in these species suggests a more complex physiological control of diapause than has been found by Williams in Platysamia cecropia. In this silkmoth the diapausing/

diapausing pupal brain does not stimulate hormonal secretion in the prothoracic glands and development is arrested. (Williams 1946, 1947). After chilling the brain resumes electrical and hormonal activity, secretion is induced in the prothoracic glands and development proceeds (Van der Kloot 1955, Williams 1956).

Bombyx mori is ~~the only~~ another insect in which the internal mechanisms are known in detail. The arrest of development is associated not with a hormonal deficiency, but the presence of a diapause factor or hormone, secreted by the maternal suboesophageal ganglion, under control of the brain. (Fukada 1951a, 1951b, Hasegawa 1951). Farther investigation showed that diapause was regulated by the balance of two hormones, the growth inhibitor from the suboesophageal ganglion and a growth-promoting hormone from the corpus allatum. (Morohoshi 1959).

The concept of antagonistic hormones is helpful in attempting to understand the complex temperature and photoperiodic relations of I.ricinus. Several alternative explanations are possible but at this stage speculation is premature since there is among the Ixodoidea little direct evidence that the brain or hormones are involved in the regulation of diapause or development. Circumstantial evidence suggests that the processes involved are probably similar to those in insects. Joffe (1964) found neurosecretory cells in the brain of I.ricinus and Hy. asiaticum P.Sch. and E.Schl., 1929 (possible synonymous with Hy. dromedarii) and identified 18 groups in the brain of D. pictus. An increase in neurosecretory material in the brain cells of Hy. dromedarii Koch, 1844, is associated with the nymphal moult, with increasing age of the adult and with feeding/

feeding. (Dhanda 1967).

Gabé (1955) studied in some detail neurosecretory activity in Ornithodoros erraticus (Lucas), 1849, O. lahorensis Neumann, 1908 and several other Arachnids. He observed that neurosecretory material from the protocerebron passed backwards along four large nerve axons to the posterio-ventrally situated 'plaque ganglionaire'. Cells in this mass secreted a product which was distinguishable from the neurosecretory material by its staining and histochemical properties. He considered it not unlikely that a hormone initiating the moult is produced by this paraganglionic mass under control of the protocerebron. No direct evidence is available although hormones are apparently involved in the moulting process in O. turicata (A. Duges), 1876. (Cox 1960).

Inactivity and delayed preoviposition in D. pictus is associated with an increase in neurosecretory material in many of the brain cell groups during the midsummer experience of hungry adults (Joffe 1965). Direct evidence awaits more refined experiments.

SUMMARY

1. There is an annual rhythm in the intensity of diapause in Ixodes ricinus and this is associated with the seasonal experience of the hungry ticks. The lower limiting temperature and the photoperiodic threshold for development of engorged ticks is high in autumn and low in spring.

2. Embryonic diapause is probably not influenced directly by the light regime.

3. Pre-feeding temperatures and photoperiods influence subsequent development. The response to constant conditions changes with the duration of exposure. Diapause intensity increases with increasing experience of high temperatures and long days; intensity decreases with increasing experience of low temperatures or short days.

4. Development proceeds without delay when a prolonged experience of short days or low temperatures is followed by a brief experience of higher temperatures and long days. Diapause is reintroduced if the high temperature and long day experience, of the unfed ticks, is again prolonged.

5. Diapause in the engorged ticks and the eggs can be broken by low temperatures. Prolonged conditioning at a short photoperiod also affects diapause intensity.

6. Photoperiodic control of diapause was also found in Ixodes hexagonus, I.canisuga, Haemaphysalis punctata and H. leporis-palustris.

7. The host seeking activity in Ixodes ricinus and the ability to feed in Dermacentor andersoni may also be influenced by the daylength.

8. The importance of temperature and daylength in the phenology of Ixodes ricinus has been discussed. Diapause in this species has been/

been compared with the phenomenon in other Arthropods.

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