Phenotypical Temperature Adaptation of Protein Synthesis in Wheat Seedlings

TIME CURVES FOR READAPTATION[1]

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ABSTRACT

Optimum temperature and temperature coefficient of protein synthesis in young wheat plants exhibit phenotypical temperature adaptation. In plants grown for 2 days at either chilling (4 C), medium (20 C), or high (36 C) temperature the respective values are: 27 C and 14.2 kilocalories per mole, 31 C and 18.2 kilocalories per mole, 35 C and 23.6 kilocalories per mole, based on in vivo [14C]leucine incorporation into total protein. The validity of the [14C]leucine incubation method has been confirmed by double-labeling experiments. Readaptation time curves are complex: the optimum temperature parameter readjusts within approximately 4 hours to an altered temperature regime, whereas the temperature coefficient needs between 4 and 96 hours for complete readaptation—depending on the temperature conditions prior to the temperature shift. Heat-preadapted plants need a recovery period at medium temperature to regain their cold adaptability with respect to optimum temperature. Cycloheximide (30 micrograms per milliliter) reduces [14C]leucine incorporation into protein by 85%, thus indicating that predominantly the cytoplasmic 80S system of protein synthesis is involved in temperature adaptation.

The present paper characterizes further the phenomenon of phenotypical temperature adaptation of protein synthesis (16), mainly by establishing detailed time curves for readaptation. These kinetic data serve a dual purpose: first, they constitute indispensable information as such; and second, they are a means to gain certain insights about the involvement or noninvolvement of different biophysical and biochemical strategies in the adaptation process. Further, it seemed important to demonstrate on at least two more or less arbitrarily selected species that the phenomenon of temperature adaptation of protein synthesis is not confined to summer wheat.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Spring wheat (*Triticum aestivum* L. var. Kolibri, 1974 and 1976 harvest) was purchased from a commercial grower (H. Rausch, Meckenheim). Seed material of *Pennisetum typhoides* was obtained from the Republic of South Africa Seedbank (Division of Seed Control), Pretoria. The sterile clone of *Lemna minor* L. was a gift from W. Huber, Technische Universität München.

Wheat seedlings were cultivated for 10 days at 20 C under continuous irradiation (150 μE. m^{-2} s^{-1}, Sylvania cool-white tubes) as described previously (16) and were then subjected to various temperature acclimation treatments. After preadaptation, the plants were harvested in batches of 20 (shoots only) and incubated with ^{3}H- or ^{14}C-labeled leucine or [^{14}C]NaHCO$_3$.

P. typhoides (Rich.) seedlings (Babala grass) were soil grown at 25 C for 10 days under the same light conditions as the wheat and then exposed to 40 and 10 C, respectively, during a 2-day acclimation period. Thirty shoots were used for each *in vivo* protein synthesis experiment.

L. minor was cultivated under sterile conditions in glass jars (15-cm diameter, 8-cm height) in 400 ml medium after Stewart (15). Cultures were diluted and transferred to a fresh medium as soon as the surface was completely covered with plants (7–10 days). Temperature and light conditions were identical with those for wheat. Logarithmically growing cultures were used for temperature adaptation experiments and exposed to 4 C (2 days), 20 C (2 days), and 36 C (12 h), alternatively. Samples for the subsequently performed [^{14}C]leucine incorporation experiments were approximately 0.5 g fresh weight.

Unless stated otherwise, uniformly labeled L-[^{14}C]leucine was used, the 1 mM solution had a specific activity of either 0.33 μCi/μmol or 0.166 μCi/μmol. The incubation procedure, the separation of the total protein fraction, and the determination of the specific activity (pmol leucine/mg protein) were outlined in detail in an earlier paper (16). In case of *P. typhoides*, an additional trichloroacetic acid precipitation step was included. The incubation solutions were used repeatedly after sterile filtration (Sartorius membrane filter, type SM 11407, 0.2 μm) until they turned brownish.

All experiments were run at least in triplicate. Regression statistics were computed, using the facilities of the Rechenzentrum of University.

RESULTS AND DISCUSSION

The validity of the data from incorporation rates of externally supplied, radioactively labeled amino acids is above all dependent upon the following conditions. First, the rate of amino acid uptake into the metabolic pool(s) is not itself the rate-limiting step for the incorporation of label into protein. Second, the swamping of the metabolic pool(s) by application of rather high exogenous concentrations—which are necessary to bring the precursor pool within the shortest possible time into equilibrium with the added label—does not *per se* affect the *in vivo* rate of protein synthesis. By the following double-labeling experiment, it could be confirmed that the temperature dependence of [^{14}C]leucine incorporation into the total protein fraction represents the μ^2 of *in vivo* protein synthesis. [^{14}C]NaHCO$_3$ (5 mCi/mmol) and [4,5-^{3}H]leucine (0.5 mCi/mmol) were offered simultaneously to wheat shoots, preadapted to 4 C for 24 h. The temperature coefficients for the incorporation of
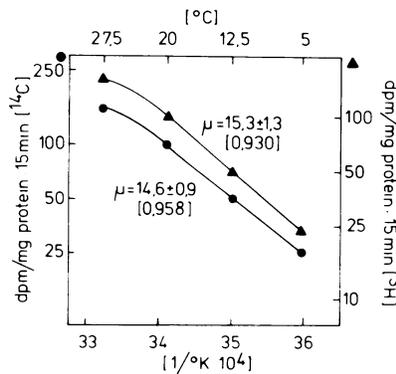
[2] Abbreviation: μ: temperature coefficient.

FIG. 1. Arrhenius plots for incorporation of label into protein via photosynthetically produced ^{14}C-amino-acids after assimilation of $^{14}CO_2$ (●) and via exogeneously supplied [4,5-^{3}H]leucine (▲).
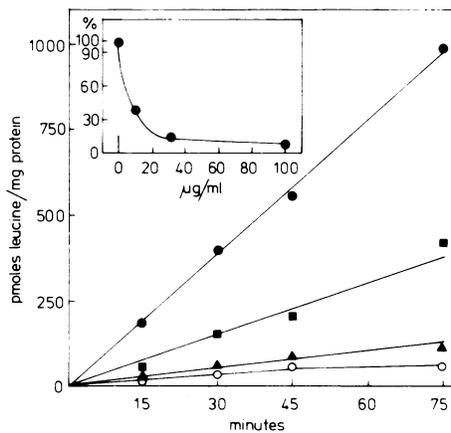

FIG. 2. Time curves for [^{14}C]leucine incorporation into protein in the presence of cycloheximide. 100 μg/ml (○), 30 μg/ml (▲) and 10 μg/ml (■); control (●). Inset: inhibition curve for cycloheximide. Wheat seedlings were 12 days old and preadapted to 20 C for 2 days.

photosynthetically produced ^{14}C-labeled amino acids and of exogenously supplied leucine into protein are almost identical (Fig. 1).

The investigation of the relative contributions of the 80S (cytoplasmic) and the 70S (plastidic and mitochondrial) protein synthesis apparatus to the total [^{14}C]leucine incorporation is the key to elucidate the question: are both systems, or only one or the other involved in phenotypical temperature adaptation of protein synthesis? Cycloheximide, known to be a specific inhibitor of 80S ribosome-directed protein synthesis, reduces leucine incorporation by 85% in concentrations as low as 30 μg/ml (Fig. 2). Hence, mainly the cytoplasmic apparatus of protein synthesis is involved in temperature adaptation. Experiments with chloramphenicol were not unequivocal; it remains uncertain if and how the 70S system participates.

The temperature characteristics of [^{14}C]leucine incorporation into the total protein fraction of wheat seedlings, having been exposed to different temperatures during a preadaptation period of 2 days, is influenced in two ways: the optimum temperature of the in vivo protein synthesis and the Q_{10} value increase markedly with rising preadaptation temperature. Plants which had been previously adapted to chilling temperatures (4 C) are characterized by a temperature optimum of 27.5 C and a Q_{10} of 2.3 (5–20 C). The corresponding values of 20 C plants[3] are 31 C and 2.9. 36 C plants exhibit the highest values for both parameters; 35 C is the

[3] Plants subjected to the standard preadaptation temperature treatment, namely 2 days at 4, 20, or 36 C are named 4 C plants, 20 C plants, and 36 C plants, respectively.

optimum temperature of in vivo protein synthesis and 4.7 the corresponding Q_{10} value (Fig. 3a and inset). The two elements of temperature adaptation complement each other with the result that 20 C plants and 36 C plants differ by about 100%, comparing their rates of protein synthesis in the temperature range = 30 C. Still larger differences exist between 4 C plants and 36 C plants. The relative contributions of the two parameters to the over-all effect of temperature adaptation change; the adaptive alteration of the Q_{10} value contributes most in the temperature region between 5 and 25 C; in the upper temperature range the shift in the optimum temperature has more influence.

A distinct discrepancy between the results reported here and the earlier ones (16) becomes evident when the data are plotted as Arrhenius curves (Fig. 3b): all values for activation energy, henceforth called "temperature coefficient" (μ), are higher by a constant percentage than the original data. Their ratio (μ 4 C to μ 20 C to μ 36 C) remained unchanged, however; no explanation can be offered.

The investigation of the time curves for the process of reacclimation from one adaptation status into another one is of interest per se; experiments of this kind may also allow, by indirect evidence, certain conclusions about the biochemical mechanisms of phenotypical temperature adaptation of protein synthesis.

Emphasizing this point, the time courses of readaptation from one extreme temperature (36 C) to the other (4 C) and vice versa were examined (Figs. 4 and 5). Both series of readaptation curves show that the shifts of μ follow a different kinetics than the alterations in optimum temperature. The former does not reach its definitive new value until 48 to 96 h after initiation of the temperature shift, whereas the latter decreases (or increases) rather rapidly and the transformation is concluded within about 4 h. The shifts of μ and the transitions of optimum temperature are summarized in Figure 6a, where the optimum temperature parameter has been replaced by the quotient of the protein synthesis rates 35 C/20 C. This term expresses fairly precisely the shifts that take place in the peak region of the Arrhenius curves.

Only 4 C plants readapt completely when the temperature is changed to the other extreme (Fig. 5); in the case of heat-preadapted plants (36 C) which were transferred to 4 C, only μ changes to the characteristic low value (kcal/mol), whereas the optimum temperature is lowered only to an intermediate level, comparable to a typical value for adaptation to 20 C. After this rapid initial decrease no further decline follows (Figs. 4 and 6a). It seems that after prolonged exposure to 36 C wheat seedlings lose part of their adaptability to cold as far as optimum temperature is concerned.
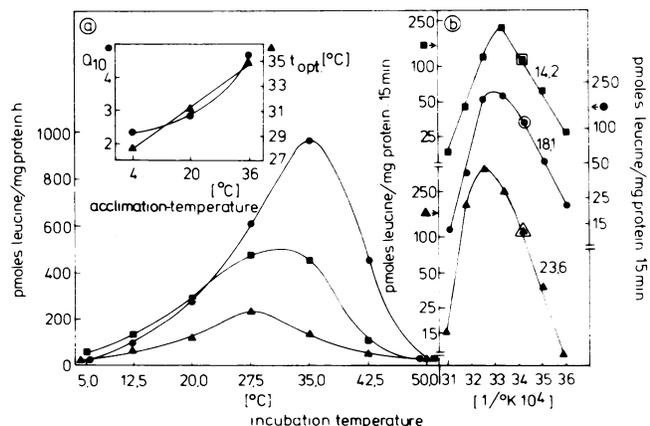

FIG. 3. Temperature characteristics for [^{14}C]leucine incorporation into protein by seedlings, adapted to 4 C (▲), 20 C (■) and 36 C (●), respectively. (a): Linear plot. Inset: Q_{10} (●) and optimum temperature (▲) as a function of acclimation temperature. (b): Arrhenius plot of the same data. Curves were standardized on 20 C (encircled values); the temperature coefficient (μ) is entered in kcal/mol.
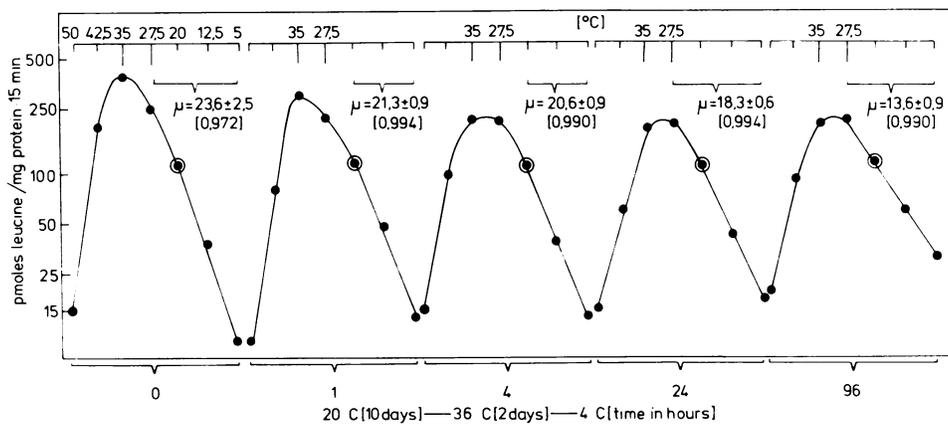
FIG. 4. Time series of Arrhenius curves for readaptation of heat-adapted wheat plants (2 days at 36 C) to low temperature (4 C). Included are the standard deviation of the temperature coefficient (μ) and the coefficient of correlation for the regression (in parentheses) computed for the linear section (bracket) of the Arrhenius curves. Curves are standardized on 20 C (encircled values).
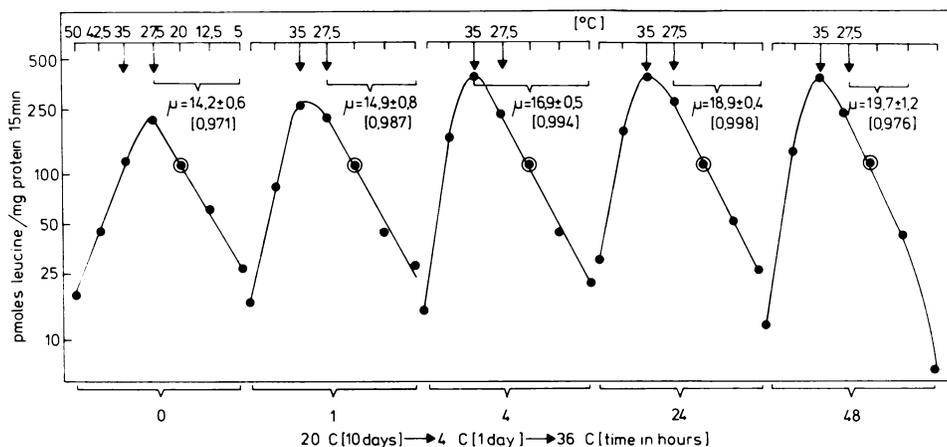

FIG. 5. Time series of Arrhenius curves for readaptation of cold-adapted (4 C) wheat plants to high temperature (36 C). For details, see legend to Figure 4.
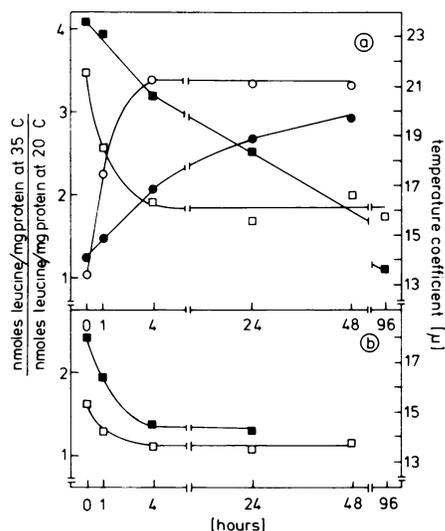

FIG. 6. a: Readaptation kinetics for temperature-coefficient (μ) (filled symbols) and for rate quotient of protein synthesis at 35 and at 20 C (open symbols) of plants that were preadapted to 4 C and transferred to 36 C (●, ○), or vice versa (■, □). b: Readaptation kinetics of plants, preadapted to 20 C and shifted to 4 C (□: rate quotient; ■ μ).

Their capability to low temperature adaptation can be restored, however, when they are exposed for an interim period to 20 C, prior to their final transfer to 4 C. Figure 7 shows that 48 h at 20 C is a sufficiently long recovery period. Only then, the temperature

optimum also reaches the low value, characteristic of cold-adapted plants, within (the subsequently applied) 4 h at 4 C. Shorter recovery periods either do not restore cold adaptability of the optimum temperature parameter at all (18 h) or are just the critical stage of recovery (24 h). After 24 h at 20 C, followed by 4 h at 4 C, the recovery process has either already taken place (Fig. 7, curve a) or atypical Arrhenius curves are obtained with inflection points at various temperatures (Fig. 7, curves b and c).

These three types of temperature curves characteristic for a 24-h recovery period are reproducible in a random mode. Plants, preadapted not to an extremely high temperature (36 C), but to a medium one (20 C), readapt quickly to 4 C not only with respect to optimum temperature, but also regarding the μ, which reaches the low value of about 14 kcal/mol just as rapidly (Figs. 6b and 8). This contradicts only at first glance the above results about readaptation from extreme temperatures. Closer inspection of Figure 6, a and b reveals that in those cases the rate of change of μ is also rather rapid during the first 4 h and corresponds well with the total change in μ in the transition from 20 to 4 C. Summing up the results about readaptation, it becomes obvious that temperature adaptation of protein synthesis is a highly complex phenomenon. The differences between μ and optimum temperature with respect to their time courses of readaptation suggest that more than one step of the protein synthesis sequence is involved. It seems remarkable that during the transitional stages of readjustment, linearity of the Arrhenius curves is always maintained (except Fig. 7, curves b and c).

The following experiment was originally designed to demonstrate the difference in the optimum temperature of protein syn-
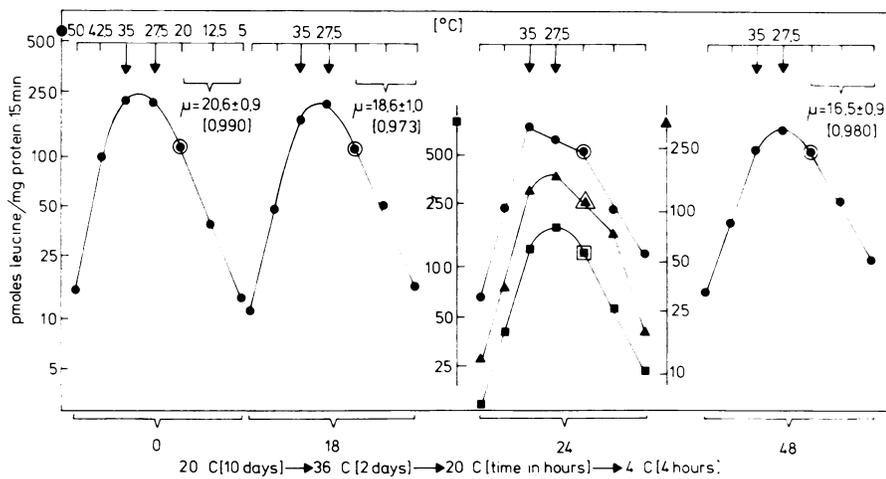
FIG. 7. Time series of Arrhenius curves for restoration of cold adaptability by a 20 C treatment of heat-preadapted plants. After 0, 18, 24, and 48 h, respectively, at 20 C the seedlings were transferred to 4 C for 4 h. After a 24-h period at 20 C the curves marked a, b, or c were obtained in a random mode; each type has been reproduced at least three times. For more details, see legend to Figure 4.
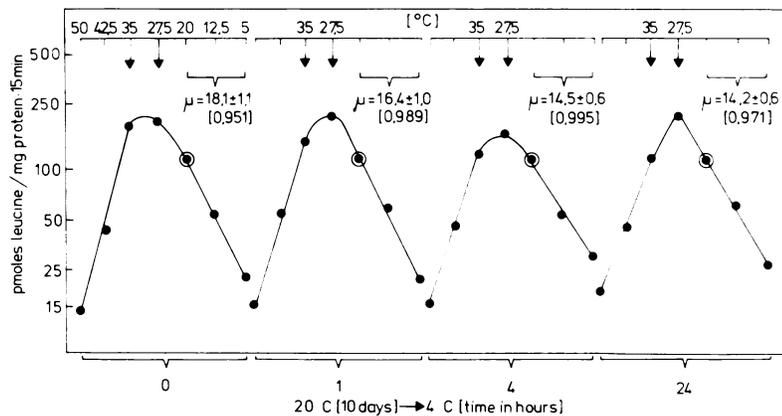

FIG. 8. Time series of Arrhenius curves for readaptation of wheat plants, preadapted to 20 C, to 4 C. For details, see legend to Figure 4.

thesis of plants adapted to different temperatures, independent of Arrhenius curves—thus evading the well known influence of the time factor on the determination of temperature optima. It turned out, however, to be an excellent demonstration of the rapidity of readaptation of the optimum temperature parameter as well. Taking it as a fact that 35 C is the optimum temperature for heat-adapted plants and at the same time supraoptimal for 20 C plants and even more so for 4 C plants, one should expect only for 36 C plants a linear rate of [^{14}C]leucine incorporation into protein at 35 C. Plants adapted to moderate or chilling temperatures should exhibit a gradually decreasing and eventually ceasing ^{14}C incorporation. Indeed, this is the result (Fig. 9). In heat-adapted plants a constant net rate of protein synthesis occurs over 90 min, whereas it stops in 4 C plants and 20 C plants after about 30 min. However, the halt in [^{14}C]leucine incorporation is only a temporary one. Protein synthesis is resumed shortly thereafter with a linear rate. This means that readaptation occurs, at least partially, within less than 1 h.

A readaptation process which takes place within 1 or a few hours after a temperature shift has been initiated can hardly be based on *de novo* synthesis of certain enzymes and/or protein factors of the protein synthesis apparatus which exhibit altered temperature characteristics compared to the respective enzymes which were present under the foregoing temperature regime (2, 3, 7, 12, 13).

Much more rapid are those adaptive responses to temperature shifts which are based upon conformational changes in preexisting enzymes (4, 6, 8–20, 14). Should conformational alterations of protein 3 C and 4 C structure due to a direct influence of ΔT on
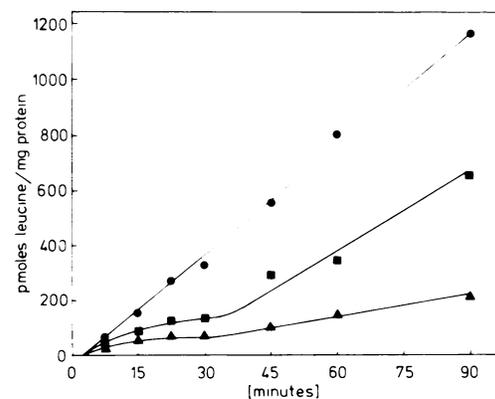

FIG. 9. Time curves for [^{14}C]leucine incorporation into protein at 35 C (incubation temperature). Wheat seedlings were 12 days old and pre-adapted for 2 days to 4 C (▲), 20 C (■), and 36 C (●), respectively.

weak forces of multistable proteins (1, 11) be responsible for thermal modulation of enzyme metabolite interactions, then one would expect this type of temperature adaptation to occur nearly instantaneously. In that case though, it would even escape detection in the experimental set-up of our *in vivo* protein synthesis studies characterized by a 15 min- ^{14}C incubation period. Conformational modulation of certain enzymes of the protein synthesis apparatus may be a delayed consequence of temperature-induced alterations of the microenvironment (5).

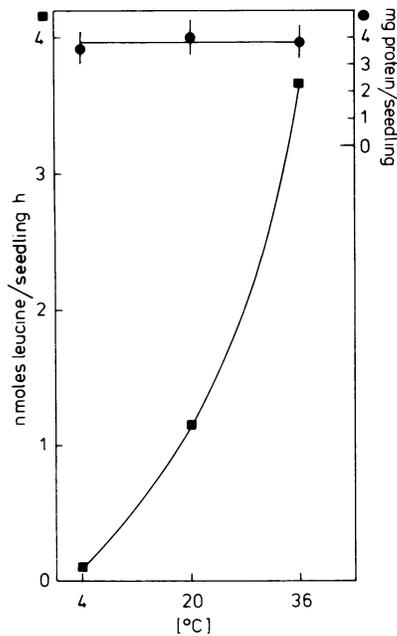Figure 10 shows that within the limitations of the method

FIG. 10. Absolute rates of protein synthesis ([^{14}C]leucine incorporation into total protein) for the three standard adaptation temperatures (4 C, 20 C, 36 C) (■) and the corresponding protein contents (●). Incorporation rates based on data from Figure 3a.
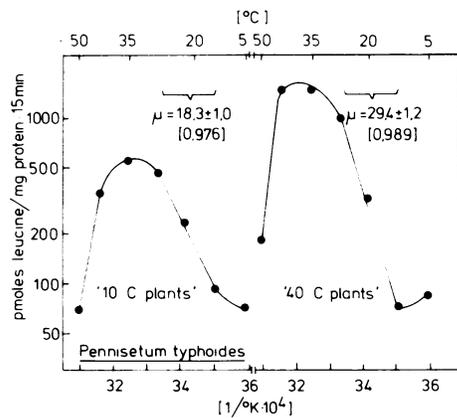

FIG. 11. Phenotypical temperature adaptation of protein synthesis in P. typhoides (Rich.). Arrhenius curves of [^{14}C]leucine incorporation into total protein for plants, preadapted for 2 days to low (10 C) and high (40 C) temperature, respectively.

employed for determining protein contents, the total amount of protein is almost identical for wheat seedlings adapted to chilling, moderate, or high temperatures despite the tremendous differences in the corresponding rates of net protein synthesis at 4, 20, and 36 C. From the data of Figure 3b a ratio of 1:12:36 can be computed for the respective rates. Therefore, it must be assumed that protein degradation between 4 and 36 C follows a temperature curve, which is a counterpart with reversed signs to the curve depicted for absolute rates of protein synthesis (Fig. 10).

The adaptive increase in the rate of net protein synthesis accounts for about two-thirds of the total increase (Fig. 3); approximately only 30% is due to the inherent enhancement of the rate of protein synthesis with rising temperature—taking the temperature characteristics of 4 C plants as a basis. Assessing the physiological virtue of the observed adaptive regulation of protein synthesis, it can be said that it adds a high amount of flexibility to the control of protein turnover.

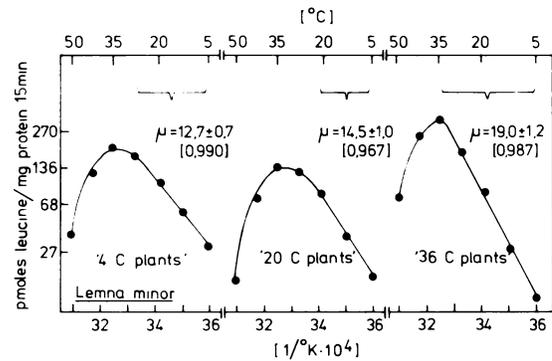Regarded superficially, phenotypical temperature adaptation of

FIG. 12. Phenotypical temperature adaptation of protein synthesis in L. minor L. Arrhenius curves of [^{14}C]leucine incorporation into total protein for plants, adapted to 4, 20, and 36 C, respectively. Preadaptation time was 12 h for heat adaptation, otherwise 2 days.

protein synthesis must be classified as a paradoxical adaptation, exhibiting an adaptive rate acceleration instead of retardation with rising temperature (17). In context with protein turnover, however, this adaptation process is not at all inversely compensatory, but on the contrary compensates heat-enhanced protein degradation. Turnover rate measurements for different adaptation conditions will have to confirm this interpretation.

Protein synthesis rates, beyond a narrow temperature range in the direct proximity of the prevailing adaptation temperature, are of little significance because readjustment of the temperature curves to a changed temperature regime takes place so fast. Heat-adapted plants, for instance, benefit only from the shift of the temperature optimum to 35 C, whereas the position of the temperature optima is quite insignificant for 20 C plants and even more so for 4 C plants. For 4 C plants it is very important that the temperature coefficient decreases by about 40% during cold acclimation, thus preventing the rate of protein synthesis from declining to practically zero level.

Is phenotypical temperature adaptation of protein synthesis confined to T. aestivum, or is it a quite widespread phenomenon? Figure 11 exhibits a basically similar pattern of adaptive change of the temperature coefficient (μ) for P. typhoides and, although less pronounced, also for the optimum temperature of [^{14}C]leucine incorporation. An explanation for the deviation from linearity of the Arrhenius curves at low temperature cannot be given. The typical increase in μ with rising preadaptation temperature can also be observed in L. minor (Fig. 12) paralleled by an extension of the linear section of the Arrhenius curves. These data for one species closely related to wheat, and a second one which occupies a rather distant systematic position suggest that phenotypical temperature adaptation of protein synthesis is probably of general importance.

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