flatfish (*Pseudopleuronectes americanus*). Flatfish are largely absent from Plymouth Harbour during July and August[7], when they move offshore into deeper, cooler water[8]. Usage of the study sites by horseshoe crabs did not correspond to invertebrate losses, based on a Spearman rank correlation across the 18 out of 26 0.5-hectare sites where crabs occurred. Mortality at sites used heavily by shorebirds exceeded mortality at less used sites.

Mortality in both years depended on the rank abundance of prey (Table 1). Higher mortality occurred in the initially more abundant prey at a site even though the ranking of individual species in July differed from site to site. Of the numerically dominant macrofauna only *Gemma gemma* did not show substantial losses when abundant. This small (< 4 mm), thick-shelled clam is the commonest species at many sites but it occurs at a low frequency in the gizzards of shorebirds, which suggest that it is relatively unpalatable.

Table 1 Mortality of intertidal invertebrates fed on by migatory shorebirds in Plymouth and Kingston Harbors in 1976

Species rank within sites in July	1	2	3–5	>5
Average mortality (1−final/initial)	0.84	0.78	0.67	0.36
Average initial density (organisms per 10 cores)	90.9	26.0	26.3	14.1
No. of 0.5 hectare areas	26	26	26	26
No. of different species	6	11	21	32

Mortality is the difference between counts at a site in mid-July and mid-September, divided by the initial count and averaged over sites. All cores were 10 cm in diameter and 10 cm deep. Variation in mortality with frequency was analysed by grouping species according to their rank in July in 10 cores from an area of one-half of a hectare. The change in density within each rank is statistically significant ($P < 0.05$). Changes in mortality between adjacent rankings are all significant at $P < 0.01$, based on a test of the equality of proportions.

Frequency dependent losses resulted in an equalisation of prey numbers during the summer. Equitability ($J' = H'/\ln(s)$, where s is the number of species and H' is the Shannon–Weaver index of diversity[9]), increased significantly from July to September of 1976 ($P < 0.001$), based on a nonparametric Wilcoxon signed ranks test[10]. This increase remains significant when the negative association between equitability and collection size has been removed by regression. A similar increase in equitability occurred in 1975. The coefficient of variation[10], another measure of dispersion, decreased significantly in both years.

Shorebirds reduce variation in the relative abundance of their prey by selective removal of numerically dominant species. This would tend to halt the continued expansion of dominant species, since few adult prey escape predation and since these prey reproduce only during the spring and summer. However, differences in palatability make it unlikely that shorebirds could by themselves stabilise an entire community of intertidal invertebrates.

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Separation of seed development from monocarpic senescence in soybeans

MONOCARPY, a single flowering phase followed by senescence and death, is a widespread phenomenon in seed plants, particularly field crops, whose large monocultures put on a dramatic display during their senescence phase[1–4]. In many, but apparently not all monocarpic species, defruiting or deflowering can prevent or at least delay monocarpic senescence and death[1–4]. For some species such as soybeans, the killing influence has been traced to the developing seeds[5]. The conspicuous correlation between the accumulation of nutrients in the developing fruit and the senescence (or apparent 'exhaustion') of the leaves (Fig. 1) has led to the sugegstion that developing fruits cause senescence by diverting or withdrawing needed nutrients or hormones from the leaves and other vegetative parts[1–4]. Here we explore the relationship between nutrient (or hormone) withdrawal or diversion and foliar senescence. Our data indicate that the development (accumulation of dry matter) of the seeds is separable from the senescence response in soybeans, and therefore the seeds may function as more than sinks.

Soybeans (*Glycine max* (L.) Merrill) cv. Anoka were grown in soil as described by Lindoo and Noodén[6]. During the first 4 weeks, the plants were kept in a greenhouse with supplementary light at night and thereafter in environmental control chambers under short (10-h) days. Foliar senescence (yellowing) and fruit development (growth and colour) were measured quantitatively by rapid, non-destructive, visual procedures, which have been checked against the more traditional (but destructive) methods for measuring senescence and fruit development described in detail elsewhere[6]. Fruit development is expressed as fruit maturity index (FMI), which reflects the average state of fruit development based on numerical values 1 (least mature) to 5 (most mature).

Our earlier studies[5] on soybeans showed the influence of the developing seeds on foliar senescence moves in a restricted pattern within the plant, thereby distinguishing it from the flower-inducing hormone, the movement of which is not as restricted and differs in other ways[7].

Single axis soybean plants with pods only on the bottom half and foliage only on the top half show greatly reduced foliar senescence compared with unmodified plants and that which occurs is primarily in the leaves nearest to the pods (Fig. 1*a*). The seed yield of the pod-bearing sections of these plants relative to unmodified plants is not reduced and is, if anything, slightly increased (unpublished data). Subsequent depodding of these 'half-and-half' plants at various stages of fruit development causes an additional reduction in senescence depending on when the pods are removed. Early (up to short day 58) removal of the pods from the bottom half stops foliar senescence, and removal at short day 71 or later does not substantially delay that senescence which does occur.

Figure 1*b* shows the time course for seed growth (dry weight per plant) and the total seed weight at the depodding. It is important to note that the dry weight of the seeds is nearly maximum (90%) at short day 66 but comparing Fig. 1*a* with *b*, it can be seen that depodding, nevertheless, causes an almost total suppression of foliar senescence. These data suggest not only that seed growth can occur without producing foilar senescence, but the senescence is induced mainly during the final phase of seed development when nutrient accumulation is probably complete. The

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spatial separation of leaves and pods seems to be important here, for normal plants with leaves subtending the pods do not show this clear distinction between seed growth and senescence when depodded at various stages of seed growth[5].

When plants are modified to a single leaf and a single pod cluster separated by two nodes, the foliar senescence pattern differs strikingly, depending which is on top, the leaves or the pods (Fig. 2). When the pods are on top, the leaf frequently senescences, whereas it does not when the pods are below the leaf. This is consistent with our earlier observation that the senescence signal tends to move downward if at all[5]. Neither delayed fruit development nor reduced seed yield can account for the lack of senescence when the single leaf is on top, the time course of fruit maturation in these modified plants is only 4–10 d behind the unmodified control (podded) plants; the yield per node is actually greater in the plants with a single pod cluster than in the unmodified control (Table 1); and the yield per node is the same whether the pod-bearing node is above or below the leaf (Table 1). Complete defoliation at FMI 1.5 reduces the seed yield in these single pod clusters by 80%; most of that seed growth which does occur apparently occurs before defoliation. The single remaining leaf seems to supply required nutrients for the growing seeds and to be subject to whatever nutrient deprivation is created by the seeds.

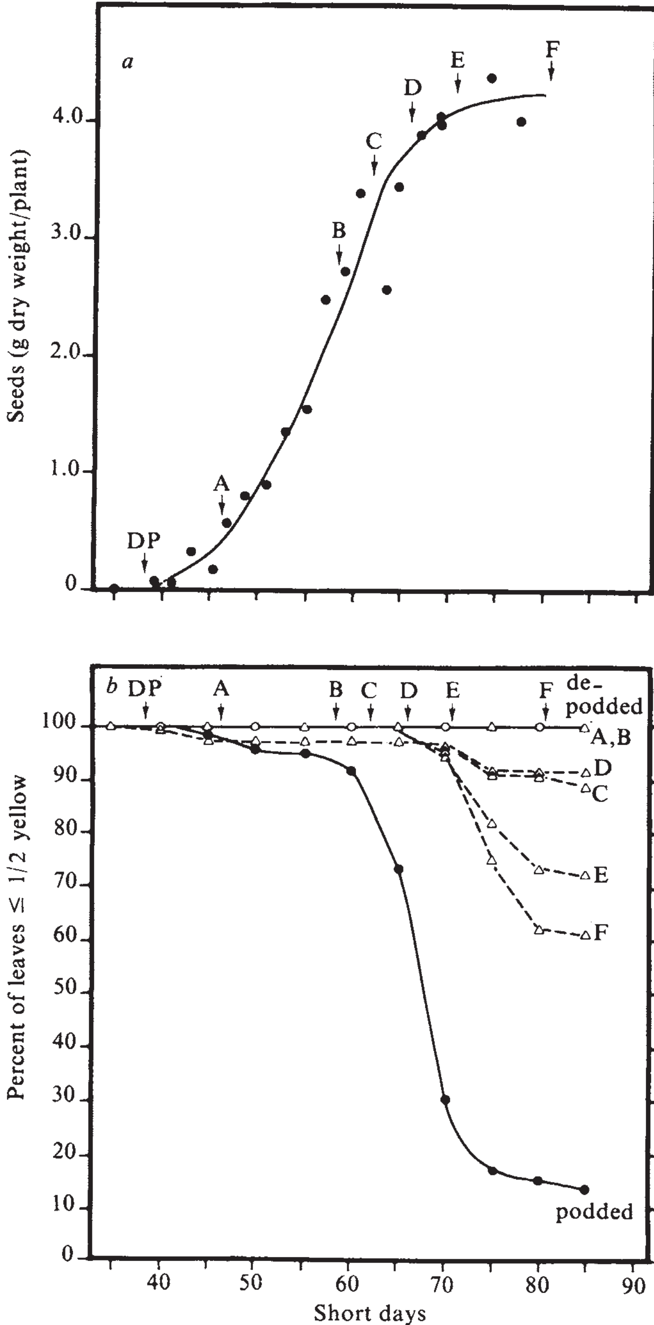

Fig. 1 *a*, Time course of seed growth in surgically modified, single-axis soybean plants (leaves only on top half, pods only on bottom half) and the effect of removing the remaining pods at various stages (indicated by A, B, C, and so on) of seed growth. Surgical modification at FMI 1.4 with subsequent removal of pods as they reached the start of pod fill, stage 2. Measurement of senescence, fruit development and FMI are explained in ref. 6. A single node between the top and bottom halves was both depodded and defoliated. *b*, Time course of foliar senescence in single-axis soybeans with leaves only on the top half and pods only on the bottom half. Same plants as in (*a*). Plants designated 'depodded' were completely depodded at FMI 1.4, whereas the 'podded' plants were not modified at all.

The experiments shown in Figs 1 and 2 show in different ways that seed development (and therefore nutrient deprivation) can occur without monocarpic senescence.

The reproductive sink size (seed yield) can be controlled by removing all except a defined number of pods from plants at FMI 1.9 (Fig. 3). In this variety and in the conditions used, seed yield (dry weight) was nearly directly proportional to the number of pods per plant; there was no pronounced tendency for seed size to increase in compensation for a reduced number of seeds. The seed nitrogen per plant is likewise proportional to the number of pods, yet foliar senescence is maximum when the number of pods is only about 40% of the maximum. A direct proportionality between sink size and rate of senescence would be expected if monocarpic senescence were caused by nutrient deprivation; in particular, the saturation of senescence induction at very low pod doses is inconsistent with the nutrient deprivation theories.

The possible explanations for the correlative influence of the developing seeds on foilar senescence fall into four

Fig. 2 Time course of foliar senescence and fruit maturation for single-axis plants pruned to one leaf and one pod cluster (separated by three nodes) compared with unmodified plants and depodded plants with all leaves intact. Depodded and defoliated at FMI 1.5 with subsequent removal of pods as they reached stage 2.

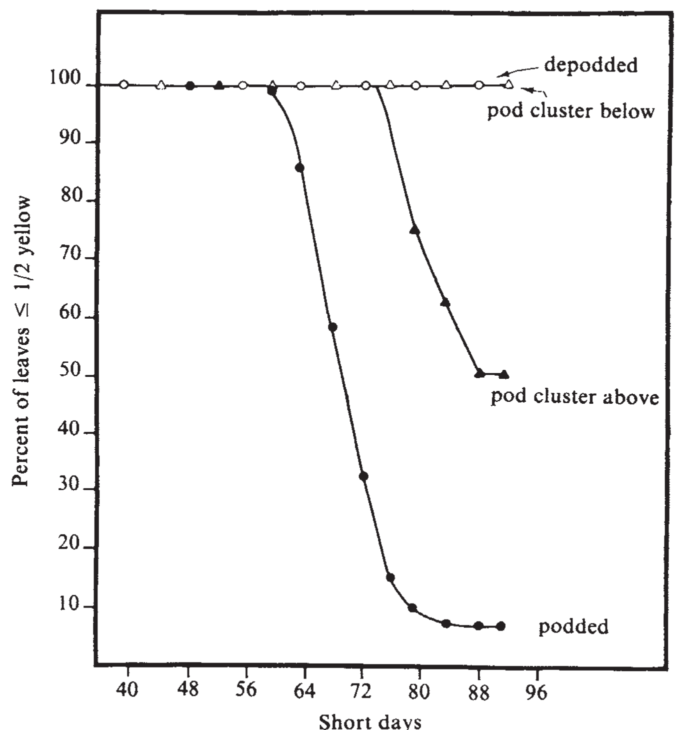

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Table 1 Seed yields on single-axis soybeans with only one leaf and one pod cluster* or without modification

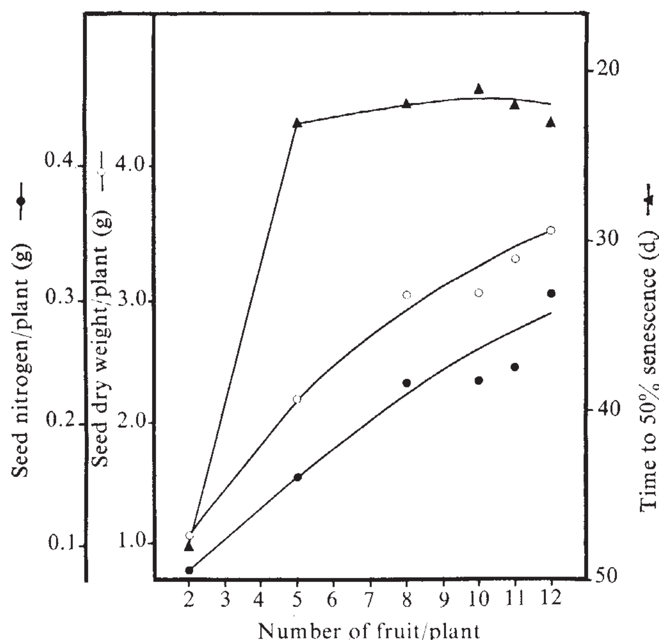
	Seeds (g dry weight/node)	
	Unmodified plant	Modified plant*
One pod cluster three nodes above a single leaf (or a pod cluster on a comparable node of an unmodified plant)	1.6	1.5
One pod cluster three nodes below a single leaf (or a pod cluster on a comparable node of an unmodified plant)	0.9	1.9

*Same plants as in Fig. 2. Unmodified plants yield about 8.3 g of dry seeds each.

general categories, which have some variations and combinations[4] but can now be evaluated in the light of the data for soybeans. The first, and least precisely defined, holds that the plants simply have a limited lifespan which runs out about the time the seeds develop. One variant of this hypothesis invokes a limited lifespan for the meristems, the loss of which causes death. Although it may be true that the shoot apical meristems degenerate during flower development thereby limiting vegetative growth (this point needs study in soybeans), depodding of soybeans at an early stage can prolong the life of these plants way beyond normal, possibly indefinitely, even though they undergo little or no vegetative growth. Thus the senescence and death we have observed in conditions favourable for soybean growth is coupled to seed production and not time or meristem degeneration.

The second hypothesis has the developing fruit diverting nutrients (for example, minerals from the roots, photosynthetic assimilate from the leaves) or hormones (cytokinins from the roots) away from the leaves thereby causing their death. Diversion of photosynthetic assimilate from the leaves seems an unlikely cause, for mature or senescent leaves seem to produce more than they need[10,11]. The diversion hypothesis, particularly the diversion of substances from the roots, is difficult to reconcile with the limited

Fig. 3 Relationship between sink size (pod number) and rate of foliar senescence. The pods were pruned to the designated number at FMI 1.9, and thereafter extras were removed as they reached stage 2. The average number of pods on unmodified plants was 14. Total nitrogen was measured by digesting samples at 280 °C in concentrated H_2SO_4 containing $CuSeO_4$ catalyst followed by dilution with H_2O and addition of Nesslers reagent for colorimetric determination[8,9].



movement of the senescence 'signal' from the seeds[5]. If such a diversion were important, then why does the single pod cluster cause senescence of the single feeder leaf only when the feeder leaf is below the pods and not when above? One would expect the pods to divert substances from the roots more effectively when they are below the leaf. Furthermore, the idea that foliar senescence is triggered by a reduction in the supply of substances produced by the roots, is also countered by the observation that foliar senescence occurs in these plants where the leaf- or pod-to-root ratio is greatly reduced. In addition, the delayed depodding of single-axis plants with pods only on the bottom half and leaves only on the top half suggests that senescence induction by the seeds may occur after most of the seed growth and its concomitant nutrient demands are complete. Note that in order to prolong the life of the plants and to maintain their productivity; expert gardeners take care to remove the fruits from certain varieties of cucumbers and beans after they fill out but before their colour changes (for example, after drain or diversion is completed but before monocarpic senescence is initiated). Finally, the sink size (pod dose or seed yield) does not parallel foliar senescence; the senescence response is saturated at a level far below the maximum level of dry weight and nitrogen accumulation in the seeds.

The third hypothesis involves nutrient drain; the developing fruit are supposed to withdraw nutrients from the leaf to the point of killing them. Indeed, there probably is some exodus of certain nutrients (particularly nitrogen) from senescing leaves[4]. The arguments against diversion, however, apply equally well to drain.

The fourth explanation gives the developing fruit or seeds a more active role: production of a substance(s) which travels out to the leaves to cause senescence and ultimately the death of the whole plant.

Although drain and diversion may be involved in the monocarpic senescence of soybeans, it seems unlikely that developing seeds exert such remarkable correlative influences simply by functioning as passive sinks, just as shoot apices do not exert apical dominance solely through nutrient diversion. Such a hormone(s) will, of course, remain hypothetical unless actually isolated and shown to travel from the source (the seeds) to the target (leaves and other parts).

Since monocarpic senescence must have evolved independently in a wide range of groups, it may well be caused by different mechanisms in other species. Indeed, the flowers of male spinach induce monocarpic senescence, even though they do not produce seed[2]. Deflowering retards this senescence, and neither nutrient drain nor diversion seem to be causal. On the other hand, it has been reported that defloration of cocklebur does not even delay the senescence and death of the whole plant[12].

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Regulation of RNA synthesis in early germination of isolated wheat (*Triticum aestivum* L) embryo

THE change from dormancy to germination of seeds requires water and an appropriate temperature. Wheat embryo germination is characterised by an initial increase in fresh weight, followed by a 5-h lag[1]. Although RNA synthesis is one of the earliest biologically measurable activities in wheat embryo germination[2,3], its mode of regulation has not been established. Reports[3–6] have suggested that the rate of synthesis does not change during the first 6 h of germination. But no adjustment was made for the specific activities of ribonucleoside triphosphate, UTP. This would have led to a misinterpretation of incorporation data when there was either a change in the concentration of the nucleotide or a difference in the rate of phosphorylation from nucleoside to nucleotide as germination progressed. To investigate further whether there is a change in the rate of RNA synthesis in the germination phase, we have used recently-developed enzyme assays[7–9] to measure picomole changes in purine pyrimidine ribonucleotide levels. We found a threefold increase in the rate of RNA synthesis of wheat embryos germinated for 40 min–5.5 h. We used the reaction catalysed by uridine 5′-diphosphoglucose pyrophosphorylase to determine the content of UTP. The key step in this analysis is the selective adsorption of the reaction product, UDP-^{14}C-glucose, on to activated charcoal in the presence of 0.8 M Trizma base[9].

In a 15-min pulse-labelling experiment, a 5.1-fold increase in the rate of ^{3}H-uridine incorporation into RNA was observed in wheat embryos germinated for 40 min–5.5 h (Table 1, experiment 1). When incorporation data were corrected by adjusting for the specific activity of the UTP, there was a 2.6-fold increase in ^{3}H-uridine incorporation into RNA during the germination phase. When germinating wheat embryos were pulse-labelled for 15 min longer followed by RNA isolation and purification, there was a 2.9-fold increase in 5.5 h (Table 1, experiment 2), similar to the 2.6-fold increase of experiment 1. These results show that the rate of RNA synthesis increases threefold during the period of germination between 40 min and 5.5 h. These results are in contrast to the idea that RNA synthesis does not change during the first 6 h of germination[3–6].

The rate of RNA synthesis in the early stage of the germination phase could be limited by either (1) RNA polymerase activity; (2) changes in the template activity of chromatin, or (3) changes in purine and pyrimidine ribonucleoside triphosphate levels. Possibilities (1) and (2) are less likely because the extraction and assay of RNA polymerase does not change for the first 6 h[11], and Yoshida and Sasaki[12] reported that the template activity of chromatin is essentially constant for the first 18 h of germination.

There is the following support for possibility (3). To explain the difference in RNA-synthesising capacity between embryos germinated for 40 min and those germinated for 5.5 h, the concentrations of purine and pyrimidine ribonucleoside triphosphates were measured. We found that the cellular level of ATP and GTP increased 60% and 30%, respectively. This minor increase in purine ribonucleoside triphosphates does not corroborate the threefold increase in the rate of RNA synthesis during this period. However, when the pyrimidine ribonucleotide pool was assayed, there was a 400% increase in the level of pyrimidine ribonucleotides during the period between 40 min and 5.5 h of germination (Table 2). The increase of UTP was similar to that of CTP. Thus this large increase in the substrate levels of UTP and CTP could account for

Table 1 Rates of RNA synthesis in wheat embryo during the germination phase

Experiment 1	Labelling periods	
	40–55 min	5.5–5.75 h
5% TCA-insoluble radioactivity	4,130±176 c.p.m. (1)	20,900±377 c.p.m. (5.1)
Specific activity of UTP	1,788±71 c.p.m. nmol^{-1}	3,511±211 c.p.m. nmol^{-1}
Adjusted incorporation	(1)	(2.6)
Experiment 2	30–60 min	5.25–5.75 h
RNA fraction	20,410±1,160 c.p.m. (1)	115,040±5,640 c.p.m. (5.6)
Adjusted incorporation	(1)	(2.9)

To prepare the trichloroacetic acid (TCA)—insoluble extract, isolated wheat (*Triticum aestivum* L. variety Fortuna) embryos (50 mg) were germinated in a Petri dish (60×15 cm) on four layers of 4.25-cm diameter Whatman No. 1 filter paper with 2 ml of water (containing chloramphenicol, 20 μg ml^{-1}) in the dark at 25 °C for 40 min or 5.5 h. The top layer containing the embryos was removed, blotted and placed in 0.5 ml of water containing chloramphenicol (20 μg ml^{-1}) and 50 μCi of purified 5-^{3}H-uridine (25 Ci mmol^{-1}) for 15 min at 25 °C. The paper was blotted and the embryos were rinsed with water. Embryos were ground first with 0.5 ml of 10% TCA and then with 5 ml of 5% TCA. The homogenate was centrifuged for 10 min at 23,500*g* and the supernatant was kept. The pellet was rinsed three times by suspending in 5 ml of 5% TCA with a motor-driven Teflon homogeniser and centrifuging for 5 min at 23,500*g*. The pellet was then suspended in 1 ml of 0.1 N NaOH and counted in 10 ml of Triton X-100 toluene-based scintillation fluid. The TCA-soluble extract was prepared as before[8]. Unlabelled UTP (1 μmol) was added to the extract and concentrated down to dryness at 40 °C in a flash evaporator. A quarter of the sample was spotted on a PEI-cellulose TLC (Brinkmann) which was then developed in either 0.5 M KH_2PO_4, *p*H 4.5, or 0.4 M LiCl, *p*H 5.5. The UTP region on the PEI-cellulose was removed from the TLC sheet by treating with 1 ml of 2 M LiCl. The suspension was then mixed with 10 ml of liquid scintillation fluid (ACS™, Amersham/ Searle). The endogenous UTP was determined as before[14]. To prepare the RNA fraction, 50 mg of isolated wheat embryos was germinated as before and labelled for 30 min with 70 μCi of 5-^{3}H-uridine. The embryos were ground first with 0.5 ml of high salt buffer (1 M KCl, 100 mM Tris-Cl, *p*H 7.6, and 5 mM EDTA). Sodium dodecyl sulphate buffer (3 ml of 1% SDS, 10 mM Tris–HCl, *p*H 7.6, and 5 mM EDTA) was added and the mixture was vortexed for 2 min. It was then extracted with water-saturated chloroform: phenol (1:1, v/v). The aqueous fractions were combined, mixed with two volumes of absolute ethyl alcohol and stored overnight at −20 °C. The precipitate was centrifuged at 23,500*g* for 20 min, rinsed three times with 5 ml of cold absolute ethyl alcohol, and then dissolved in 1 ml of 0.5% SDS buffer (0.5 M NaCl, 10 mM Tris–HCl, *p*H 7.6, 0.5% SDS and 1 mM EDTA). Sample of 40 μl was mixed with 10 ml of liquid scintillation fluid (ACS™) and counted as before. The data represent an average of duplicate samples. Numbers in parentheses signify the fold increase of the 5.5-h values above those of the 40-min values.

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