hatched chick is in a state of pronounced nutritional fasting, with a predominantly lipid metabolism similar to that found after a 72-h fast[15]. The cerebral arteriovenous difference of 3-hydroxybutyrate is 6–9 times as high in the 1- as in the 4- and 30-day-old chick respectively (table). The very active uptake of 3-hydroxybutyrate by the brain of the newly-hatched chick is paralleled by the high activity of 3-hydroxybutyrate dehydrogenase in the telencephalon of the young chick (fig.). 3-Hydroxybutyrate is a very effective precursor for amino acid biosynthesis in the brain of the 1-day-old chick[8] and it appears to be at least as important as glucose as a source of metabolic fuel in the brain of the newly-hatched chick.

In the rat, the blood content of 3-hydroxybutyrate is 2–3 times lower than in the chick whereas that of acetoacetate is nearly 10 times as high in the suckling rat as in the 1-day-old chick[3,17,21]. These very important differences in blood ketone body concentrations in both species are linked to the high activity of hepatic 3-hydroxybutyrate dehydrogenase in the rat compared to the chick[19,22]. The cerebral arteriovenous difference of 3-hydroxybutyrate content in the 16–20-day-old rat is twice as low as in the 1-day-old chick[4]. The intense utilization of 3-hydroxybutyrate by the brain of the suckling rat is, as in the young chick, linked to the high activity of the enzymes regulating ketone body metabolism[3,17,23], 3-hydroxybutyrate being a very effective precursor for the biosynthesis of amino acids[5,6] and lipids[24] in the brain of young rats.

In conclusion, as in the brain of the suckling rat, 3-hydroxybutyrate represents a major metabolic fuel for the chick telencephalon at hatching time when the chick is in a state of pronounced nutritional fasting characterized by a predominantly lipid metabolism and a reduced supply of glucose.

1 Thanks are due to Mrs C. Math for illustrating the manuscript. This research was supported by grants from the Centre National de la Recherche Scientifique (E.R.A. 331) and from the Fondation pour la Recherche Médicale.

2 H.A. Dymsza, D.M. Czajda and S.A. Miller, J. Nutr. *84*, 100 (1964).
3 H.A. Krebs, D.H. Williamson, M.W. Bates, M.A. Page and R.A. Hawkins, Adv. Enz. Regul. *9*, 387 (1971).
4 R.A. Hawkins, D.H. Williamson and H.A. Krebs, Biochem. J. *122*, 13 (1971).
5 D.C. De Vivo, M.P. Leckie and H.C. Agrawal, Brain Res. *55*, 485 (1973).
6 D.C. De Vivo, M.P. Leckie and H.C. Agrawal, J. Neurochem. *25*, 161 (1975).
7 R.L. Hazelwood, in: Avian Biology, vol.2, p.471. Ed. D.S. Farner, J.R. King and K.C. Parkes. Academic Press, New-York 1972.
8 A. Nehlig, P.R. Lehr and J. Gayet, Comp. Biochem. Physiol. *61B*, 59 (1978).
9 P.R. Lehr and J. Gayet, Comp. Biochem. Physiol. *53B*, 215 (1976).
10 H.U. Bergmeyer and E. Bernt, in: Methods of enzymatic Analysis, vol.3, p.1205. Ed. H.U. Bergmeyer. Academic Press, New York 1974.
11 J. Mellanby and D.H. Williamson, in: Methods of enzymatic Analysis, vol.4, p.1840. Ed. H.U. Bergmeyer. Academic Press, New York 1974.
12 D.H. Williamson and J. Mellanby, in: Methods of enzymatic Analysis, vol.4, p.1836. Ed. H.U. Bergmeyer. Academic Press, New York 1974.
13 O.H. Lowry and J.V. Passonneau, A flexible System of enzymatic Analysis. Academic Press, New York 1972.
14 A.F.W. Hughes, Phil. Trans. R. Soc. *224B*, 75 (1934).
15 P.S. Belo, D.R. Romsos and G.A. Leveille, J. Nutr. *106*, 1135 (1976).
16 L.J. Brady, D.R. Romsos, P.S. Brady, W.G. Bergen and G.A. Leveille, J. Nutr. *108*, 648 (1978).
17 M.A. Page, H.A. Krebs and D.H. Williamson, Biochem. J. *121*, 49 (1971).
18 G. Dahlquist, Acta physiol. scand. *98*, 237 (1976).
19 A. Nehlig, M.C. Crône and P.R. Lehr, Biochim. biophys. Acta *633*, 22 (1980).
20 J. Katz, S. Golden and M. Chenoweth, Hoppe-Seyler's Z. physiol. Chem. *357*, 1387 (1976).
21 G. Dahlquist, U. Persson and B. Persson, Biol. Neonate *20*, 40 (1972).
22 N. Latruffe, M.N. Feuvrier, N. Bichet and Y. Gaudemer, Biochimie *60*, 71 (1978).
23 C.B. Klee and L. Sokoloff, J. biol. Chem. *242*, 3880 (1967).
24 M.S. Patel and O.E. Owen, J. Neurochem. *28*, 109 (1977).

A bioluminescent assay for aldehyde sex pheromones of insects[1]

E.A. Meighen, K.N. Slessor and G.G. Grant

Department of Biochemistry, McGill University, 3655 Drummond Street, Montreal (Quebec, Canada H3G 1Y6), Department of Chemistry, Simon Fraser University, Burnaby (Canada V5A 1S6), and Forest Pest Management Institute, Canadian Forestry Service, Sault Ste. Maria (Canada), 7 November 1980

Summary. Aldehyde dependent bacterial luciferases respond quantitatively to ng quantities of aldehyde pheromones from insects. The luminescent response is the basis for a quantitative assay used to analyse sex pheromone from several sources including individual female moths.

Bacterial luciferases catalyze the oxidation of reduced flavin mononucleotide ($FMNH_2$) and long chain aliphatic aldehydes resulting in the emission of light. These luciferases are isolated from bioluminescent bacteria found either free living in the ocean or living in symbiosis with certain fish. In the in vitro biochemical reaction, light emission rises rapidly (< 1 sec) to a maximum and then decays with time. This bioluminescent reaction can be used for analyses of aldehydes since the maximum light intensity obtained is dependent on the type and amount of long chain aldehyde.

We have discovered that bacterial luciferases will also respond quantitatively to very low levels of the unsaturated aldehydes which comprise the sex pheromones of a relatively large number of insects, many of which are of major economic importance (such as the spruce budworm *Choristoneura fumiferana,* the corn earworm *Heliothis zea* and tobacco budworm *H. virescens*). A survey of the literature shows that no less than 19 species of moths in 10 families and 4 species of dermestid beetles have a least 1 component of their sex pheromones identified as an aliphatic aldehyde and the number of insects shown to have such pheromones

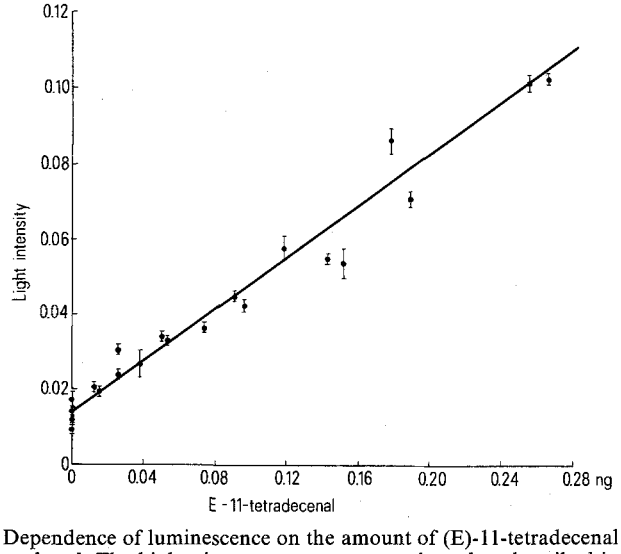
is rapidly increasing. In many cases, the minor components of these pheromones are also aldehydes. For example, the sex pheromone of the spruce budworm is a mixture of 96% (E)-11-tetradecenal and 4% (Z)-11-tetradecenal[2]. We have developed the bacterial bioluminescent assay to quantitatively measure aldehyde pheromones of insects.

In the assay, an aqueous solution of aldehyde pheromone is injected into a reaction mixture consisting of luciferase and $FMNH_2$[3] contained in a vial within a light tight cylinder. The rapid mixing of luciferase and substrates results in the emission of blue green light at 490 nm[4]. The emitted light is detected with a photomultiplier tube[5] connected to the cylinder and is graphically displayed with a pen recorder. Since the reaction and recorder response are immediate, repetitive analysis of aldehyde samples are rapidly and easily obtained.

Table 1 compares the bioluminescent response of bacterial luciferase to several aldehyde pheromones of insects. The responses are compared with the same amount of tetradecanal, the aldehyde that is believed to be the in vivo substrate for this luciferase[6–8], and which gives the maximum bioluminescent response in vitro of any saturated aldehyde at low concentration. Table 1 also shows that the luminescent response varies with the chemical structure of the aldehyde pheromone and thus may also have potential to qualitatively assess insect sex pheromones. Fortuitously, the chain length specificity of the bacterial luciferases is from 8 to 18 carbons which encompasses the chain length (12–18 carbons) of almost all aldehyde pheromones found in insects.

The high sensitivity of the bioluminescent reaction for aldehyde pheromones is illustrated in the figure by its response to very low levels of (E)-11 tetradecenal, the major component of the spruce budworm pheromone. As little as 0.02 ng (0.1 pmole) of pheromone can be quantitated using the standard curve (fig.) which is linear over a 10^4–10^5 range of pheromone concentration. The quantity of pheromone on the gland surface of individual female moths can also be detected and measured. Glands from 2-day-old female spruce budworms held in continuous light were excised and rinsed with 10 µl of heptane[10]. The solvent was evaporated off and the residual pheromone redissolved in water for analysis. An average of 2.3 ng of pheromone was detected per gland with a range of 0.2–5.7 ng (table 2). The same quantity of pheromone was found independently by analysis with capillary gas chromatography[14]. By contrast the heads and antennae of male or female budworms or of pieces of abdomen directly adjacent to the gland contained very little aldehyde (0–0.2 ng). Pheromone on the gland surfaces of individual female moths of the navel orangeworm and the corn earworm have also been successfully assayed with this technique.

Pheromone extracted from Porapak Q, a solid phase absorbent for organic compounds, can easily be measured using the bioluminescent assay. This is of significant practial importance because Porapak is frequently used to trap airborne pheromone released by calling females[13] or from



Dependence of luminescence on the amount of (E)-11-tetradecenal analyzed. The bioluminescent assay was conducted as described in table 1. The maximum luminescence is plotted vs the amount of added E-11-tetradecenal. Each point is the average of 2 assays with the bars representing the range of the experimental data. Linear regression analysis gave an equation for the line of $y = 0.01 + 0.37x$ with a correlation coefficient of 0.97.

Table 1. Bioluminescent response of bacterial luciferase to aldehyde pheromones of insects

Aldehyde pheromone	Relative bioluminescent response*	Insects**
None	< 0.2	
E-11-tetradecenal	100	Spruce budworm[2]
Z-11-tetradecenal	80	Orange tortrix moth[9]
Z-11-hexadecenal	120	Corn earworm[10], European cotton bollworm[11], Tobacco budworm[10]
E-14-methyl-8-hexadecenal	30	Dermestid beetles[12]
Z-14-methyl-8-hexadecenal	60	Dermestid beetles[13]

* The aldehyde pheromones (100 pmoles) in 1.0 ml of water at 22° were injected into 1.0 ml of 0.05 M phosphate, 0.05 M mercaptoethanol, 0.01 M NH_2OH, pH 7.0, containing *Beneckea harveyi* luciferase and 5×10^{-5} M $FMNH_2$, reduced with sodium dithionite[3]. The value for the maximum light intensities are given as a percentage of the response obtained with the same amount of tetradecanal. ** Examples of insects containing the respective aldehydes as the major component of their sex pheromone.

Table 2. Pheromone levels in the female spruce budworm moth

	Number of budworms analyzed	Average amount per budworm ng ± SD	Range (ng)
Female gland	36	2.3 ± 1.6	0.2–5.7
Female abdomen	6	0.1 ± 0.07	0.0–0.2
Female head/antennae	6	0.2 ± 0.07	0.1–0.3
Male head/antennae	6	0.1 ± 0.06	0.0–0.2

2- and 3-day-old eastern spruce budworms, *Choristoneura fumiferana*, maintained in continuous light, were excised between 15.00 and 17.00 h, E.S.T. and extracted with 10 µl of heptane for 10 min as in Klun et al.[10]. The heptane extract was directly transferred to a 20 ml glass vial, evaporated under low vacuum for 2 min to remove the heptane, vortexed for 10 sec with 10 ml of water, and then assayed 10 min later by the procedure described in table 1. The abdomen samples refer to the tissue in immediate proximity to the gland with the mass of material analyzed being approximately twice that for the gland. Each budworm sample was analyzed at least twice, the average value corrected for background response for reference heptane samples containing no budworm material and then converted into ng based on the luminescent response of standards of E-11-tetradecenal in heptane carried through the identical process.

the atmosphere[15] in a mating disruption trial. With the assay, as little as 2 ng of (E)-11-tetradecenal deposited on 200 mg of Porapak Q (pre-cleaned by repetive washing with hexane) was extracted and quantitated. A similar analysis by conventional packed column gas chromatography failed to detect the pheromone. High recoveries of pheromone (> 50%) from a stream of air were also measured by absorbing the pheromone onto Porapak Q and subsequently extracting. This technique is currently being used to measure pheromone released by female moths and from lures designed for pheromone traps. The rapidity and sensitivity of this bioluminescent assay provides a powerful new analytical tool for measuring aldehyde pheromones of insects.

1 This investigation was supported by a U.S.D.A. Forest Service Program entitled Canada United States Spruce Budworms Program (23-345) and the Medical Research Council of Canada (MT-4314).

2 C.J. Sanders and J. Weatherston, Can. Ent. *108*, 1285 (1976).
3 E.A. Meighen and R.E. MacKenzie, Biochemistry *12*, 1482 (1973).
4 J.W. Hastings, A. Rev. Biochem. *37*, 597 (1968).
5 G.W. Mitchell and J.W. Hastings, Analyt. Biochem. *39*, 243 (1971).
6 S. Ulitzur and J.W. Hastings, Proc. natl Acad. Sci. USA *75*, 266 (1978).
7 O. Shimomura, F.H. Johnston and H. Morise, Proc. natl Acad. Sci. USA *71*, 4666 (1974).
8 D. Riendeau and E. Meighen, J. biol. Chem. *254*, 7488 (1979).
9 A.S. Hill, R.T. Carde, H. Kido and W.L. Roelofs, J. Chem. Ecol. *1*, 215 (1975).
10 J.A. Klun, J.R. Plimmer, B.A. Bierl-Leonhardt, A.N. Sparks and O.L. Chapman, Science *204*, 1328 (1979).
11 B.F. Nesbitt, P.S. Beevor, D.R. Hall and R. Lester, J. Insect. Physiol. *25*, 535 (1979).
12 J.H. Gross, R.C. Byler, R.M. Silverstein, R.E. Greenblatt, J.E. Gorman and W.E. Burkholder, J. Chem. Ecol. *3*, 115 (1977).
13 J.H. Gross, R.C. Byler, R.F. Cassidy Jr., R.M. Silverstein, R.E. Greenblatt, W.E. Burkholder, A.R. Levinson and H.Z. Levinson, J. Chem. Ecol. *2*, 457 (1976).
14 P.J. Silk, S.H. Tan, C.J. Wiesner and R.J. Ross, Envir. Ent., in press (1981).
15 C.J. Wiesner, P.J. Silk, S.H. Tan and S. Fullerton, Can. Ent. *112*, 333 (1980).

The chromosomal location of phosphatase isozymes of the wheat endosperm

J. Salinas, M. Pérez de la Vega and C. Benito[1]

Departamento de Genética, Facultad de Biología, Universidad Complutense, Madrid-3 (Spain), 17 October 1980

Summary. The 7 phosphatase isozymes found in the endosperm of hexaploid wheat (*Triticum aestivum* L.) are related to chromosomes of homoeology group 4. At least 4 loci are related to phosphatase isozymes.

Hexaploid wheat phosphatase isozymes have been previously studied using the nulli-tetrasomic and ditellosomic series of the 'Chinese Spring' wheat cultivar[2,3]. The 1st work[2] reported that 35-day old euploid seedlings of 'Chinese Spring' showed 6 alkaline phosphatase isozymes (1-6 from faster to slower migration) after electrophoresis in starch gels; isozymes 1 and 2 were related to chromosome 4B, and isozymes 5 and 6 to 4D; no influence of chromosome 4A was reported. In further work[3] using a similar technique, again 6 bands of acid phosphatases were found in 7-day-old euploid seedlings, but this time the use of nulli-tetrasomic and ditellosomic strains revealed that these 6 bands were the results of the overlapping of a least 9 isozymes. Isozymes 4 and 8 were related to chromosome arm 4Aβ, isozymes 2 and 3 to 4BS, and isozymes 5 and 6 to 4DL.

The purpose of the present work was to study alkaline and acid phosphatase isozymes of hexaploid wheat endosperm, the location of the chromosome arms related to these isozymes, and the comparison of these results with previous data reported for wheat seedlings[2,3].

Materials and methods. The materials used in this study were the nullitetrasomic and ditellosomic strains of *Triticum aestivum* L. cv. 'Chinese Spring' supplied by Professor E.R. Sears. The chromosomic constitution of these nullitetrasomic and ditellosomic strains is indicated in table 1. The analysis was carried out with individual crude extracts of embryoless endosperms from dry seeds. Phosphatase isozymes were revealed by means of horizontal 10% polyacrylamide gel slab electrophoresis using buffers and staining solution previously described[2]. 2 different pHs, 8.5 (alkaline phosphatase) and 5.0 (acid phosphatase), were employed for staining.

Results and discussion. The results obtained with euploid 'Chinese Spring' (genomes A, B and D) showed an identical 7-banded pattern at both pHs (8.5 and 5.0) for endosperm phosphatases, but better results were obtained at alkaline

Table 1. The chromosomic constitution of nulli-tetrasomic and ditellosomic strains of 'Chinese Spring'

Strains	Chromosomal dosage of nulli-tetrasomics		
	4A	4B	4D
Euploid Ch.S.	2	2	2
Nulli-4B-tetra-4A	4	0	2
Nulli-4B-tetra-4D	2	0	4
Nulli-4D-tetra-4A	4	2	0
	Chromosome arms present in ditellosomics		
	4Aα	4BS	4DS
Euploid Ch.S.	4A	4B	4D
	4Aβ	4BL	4DL
	4Aα	4BS	4DS
Ditello 4Aα	4A	4B	4D
	-	4BL	4DL
	4Aα	-	4DS
Ditello 4BL	4A	4B	4D
	4Aβ	4BL	4DL
	4Aα	4BS	4DS
Ditello 4DS	4A	4B	4D
	4Aβ	4BL	-