mine, glutamate or aspartate. Ammonium acetate was supplied to a final concentration of 1.1 mg/ml and the amino acids to a concentration of 100 µg/ml. All enzyme assays were carried out using cells from the exponential growth phase. Nitrogenase activity in whole cells was determined using the acetylene reduction test according to Hardy et al.[6].

Cell-free extracts in 0.25 M sucrose solution were prepared following the rupture of cells by sonication. The extracts were clarified by centrifuging at 10,000 × g for 10 min in a refrigerated centrifuge. Glutamine synthetase (GS) was assayed by measuring the amount of γ-glutamyl hydroxamate formed in the presence of Mn^{2+} as described by Shapiro and Stadtman[7]. Glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) activities were determined by following the rate of NADPH oxidation at room temperature[8,9]. Glutamate-oxaloacetate transaminase (GOT) activity was determined colorimetrically according to Mohun and Cook[10]. Nitrate reductase (NR) was assayed according to Hageman et al.[11]. The total protein was estimated following the procedure of Lowry et al.[12].

Results and discussion. As shown in table 1, the nitrogenase activity of the wild strain was inhibited to varying degrees in the presence of different nitrogenous compounds. The GS and GOGAT activities were high while GDH activity was not detectable in the wild strain grown in nitrogen-free medium. In the presence of ammonia, although GS and GOGAT activities could still be detected, the GDH activity was found to be high in comparison with nitrogen - free medium and media with other nitrogen sources. The GDH pathway operates at high concentrations of ammonia, while the GS-GOGAT pathway operates at low concentrations[13,14]. Where GS activity was low in nitrogen-supplied cultures, the nitrogenase activity was also low because its synthesis requires active GS[15].

Table 2. Nitrate reductase activity (nmoles of nitrite formed/min/mg protein) of wild strain and the mutant

Nitrogen source	wild	nif^{-III} mutant
No nitrogen	3400	No growth
Ammonium acetate	7800	Not detected
NO_3^-	10,000	No growth

The nif^{-III} mutant could use ammonia as well as glutamate, glutamine and aspartate, but did not show nitrogenase activity in any of the media. The GS, GOGAT and GDH activities showed the same trend as in the wild strain. The GOT activity did not exhibit any significant difference in comparison with the wild strain.

The wild strain showed NR activity on media without nitrogen or with ammonia or with nitrate. The activity was increased in the presence of nitrate. The mutant had no NR activity either in the presence or absence of nitrate (table 2).

The absence of molybdenum-containing nitrate reductase and nitrogenase activites suggests that the mutation may affect the transport of molybdenum or its incorporation into enzyme protein[16]. Hence this mutant may be regarded as phenotypically I^-II^+.

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Glucose and 3-hydroxybutyrate utilization by chick telencephalon during postnatal development[1]

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Summary. The cerebral arteriovenous difference in glucose content remains constant during the whole postnatal development of the chick, whereas that of 3-hydroxybutyrate is 6-9 times as high in the 1-day-old chick as in the 2-30-day-old chick.

In consequence of the high lipid content of maternal milk[2], the infant rat develops a marked ketosis as early as birth[3] and ketone bodies - acetoacetate and 3-hydroxybutyrate - are largely utilized by the brain[4]; indeed, 3-hydroxybutyrate appears to be a very active precursor of amino acids in the brain of the 15-day-old rat[5,6]. The newly-hatched chick is in a state of pronounced nutritional starvation with high fatty acid oxidation in the liver inducing an excessive release of ketone bodies into the blood plasma. This neonatal lipid metabolism is progressively replaced by a predominantly carbohydrate metabolism during the first 5 days after hatching[7]. The 3-hydroxybutyrate is present at very high concentrations in the blood of 1-day-old chicks; it is also a very active precursor of amino acids in the newly-hatched chick telencephalon[8], while glucose is actively incorporated only towards the 4th day after hatching[9]. In order to determine the relative importance of glucose and 3-hydroxybutyrate in the metabolism of chick brain during

postnatal development and particularly during the days after hatching, we studied the contents of glucose, lactate and 3-hydroxybutyrate in chick blood between the 1st and the 30th days after hatching as well as the cerebral arteriovenous differences of these 3 metabolites during the same developmental period.

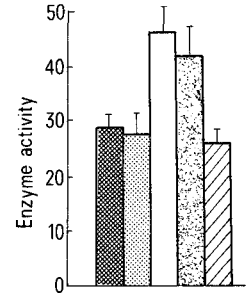
Materials and methods. The experiments were performed on chicks *(Gallus domesticus)* from the breed of Vedette J.V. 15 (block I.S.A.), at 1, 2, 4 and 30 days of age. The 1-day-old chicks were taken out of the electric incubator and, up to 30 days, the chicks were allowed free access to a standard diet containing 50% carbohydrate, 21% protein, 4.5% lipid, 4.5% cellulose, 7% minerals and 14% water supplemented with vitamins A and D_3 (Duquesne-Purina). They could have water at all times and were kept in groups in breeding cages.

The total blood was collected in a preheparinized glass dish after decapitation of the bird; then it was deproteinized by chilled 10% (w/v) perchloric acid and centrifuged at $3000 \times g$ for 15 min at 4 °C. The supernatant solution, previously neutralized by 20% KOH, was used for the enzymatic estimation of glucose[10], acetoacetate[11], 3-hydroxybutyrate[12] and lactate[13] in a Beckman spectrophotometer model Acta III. For the evaluation of cerebral arteriovenous differences, the chicks were previously anesthetized with 35% chloral hydrate (0.25 ml/100 g b.wt). Arterial blood samples (0.1–0.3 ml) from 1- to 30-day-old chicks were taken from the left ventricle of the heart. Venous blood samples (0.1 and 0.3 ml) representative for the telencephalon were obtained within 30 sec from the confluence of the middorsal cervical sinus and the middorsal cervical vein (following the terminology of Hughes[14]). Blood samples were deproteinized with 2% (w/v) perchloric acid. The samples neutralized by 10% KOH were used for the enzymatic determination of glucose, 3-hydroxybutyrate and lactate as previously described.

Results and discussion. Blood glucose content in the chick as well as the uptake of glucose by the chick telencephalon remain high and relatively unchanged during the whole of postnatal development (table). The high blood glucose content of the chick is probably related to the great capacity of this animal species for recycling tricarbon units originally derived from glucose as lactate, in order to maintain the glycemia unchanged during fasting periods which can last up to 72 h[15,16]. This is confirmed by the high lactate content in the blood of the chick, particularly after the 4th day of life. Even in the adult chick, the excess of glucose leaves the brain as lactate, the cerebral arteriovenous difference of the latter being about twice as high in the 1- as in the 2–30-day-old chick. These results confirm the predominance of anaerobic metabolism in the brain of the newly-hatched chick.

In the rat, the glycemia and the cerebral arteriovenous difference of glucose content are nearly twice as low as in the chick during the whole of postnatal development[3,17,18]. Indeed, in the rat, glucose recycling is 2–3 times less active than in the chick[18]. Moreover, in adult rats, unlike what is observed in the chick, excess glucose leaves the brain unchanged, whereas in suckling rats, a fair percentage of glucose leaves the brain as lactate and pyruvate[4,18].

The blood content of 3-hydroxybutyrate in the chick is very high, particularly in the 1-day-old chick, whereas that of acetoacetate remains very low during the whole of postnatal development. The 3-hydroxybutyrate/acetoacetate ratio in the blood reaches the value of 44 in the 1-day-old chick, then diminishes and reaches the value of 13 at 30 days and even 6 in the 5-month-old chicks[15]. The high blood content of 3-hydroxybutyrate as well as the high 3-hydroxybutyrate/acetoacetate ratio shows that the newly-



Variations of 3-hydroxybutyrate dehydrogenase activity in the telencephalon of the developing chick[19]. Enzyme activities are expressed as nmole of acetoacetate formed per min and per mg of protein ± SD. 20-day-old embryo; 1-day-old chick; 2-day-old chick; 4-day-old chick; 30-day-old chick.

Blood total, arterial and venous concentrations and cerebral arteriovenous C (A-V) differences of glucose, lactate, acetoacetate and 3-hydroxybutyrate in 1–30-day-old chicks

	Age of the chicks (days) 1	2	4	30
Glucose (mg/100 ml)				
Total blood	157± 8 (15)	164± 9 (14)	154±9 (14)**	142±12 (22)
Arterial blood	146±11 (10)	159±13 (16)	162±8 (12)	198±21 (18)
Venous blood	127± 9 (10)	143±11 (16)	142±7 (12)	177±18 (18)
C (A-V)	−19± 3 (10)	−16± 5 (16)	−20±6 (12)	−21± 8 (18)
Lactate (mM)				
Total blood	0.84±0.08 (18)	0.91±0.09 (14)***	1.34±0.15 (12)	1.34±0.15 (15)
Arterial blood	0.86±0.14 (10)	1.54±0.14 (12)	1.64±0.22 (14)	1.08±0.13 (12)
Venous blood	1.48±0.22 (10)	1.87±0.16 (12)	1.95±0.24 (14)	1.32±0.14 (12)
C (A-V)	+0.62±0.08 (10)***	+0.33±0.08 (12)	+0.31±0.08 (14)*	+0.24±0.09 (12)
Acetoacetate (mM)				
Total blood	0.051±0.009 (10)***			0.033±0.006 (27)
3-Hydroxybutyrate (mM)				
Total blood	2.27±0.51 (20)***	0.62±0.10 (17)***	0.35±0.05 (16)	0.44±0.05 (16)
Arterial blood	2.17±0.35 (16)	0.46±0.07 (10)	0.36±0.06 (11)	0.34±0.05 (10)
Venous blood	1.70±0.38 (16)	0.39±0.07 (10)	0.28±0.06 (11)	0.29±0.05 (10)
C (A-V)	−0.47±0.14 (16)***	−0.07±0.02 (10)	−0.08±0.02 (11)*	−0.05±0.02 (10)

The number of birds is given in parentheses. * $p<0.05$; ** $p<0.005$; *** $p<0.0005$.

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.

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

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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