variation of enzyme activity during the cycle of 24-h study. Maximum activity was observed at the beginning of sunset (18.00 h), when it was 2.1-fold higher than the minimum activity during the early daylight period (10.00 h) in RRIM 600 and 1.7-fold in KRS 21 (fig. 1). Figure 1 shows similar patterns for both clonal types investigated. The apparent regulation of the light-dark phase is probably due to physiological processes associated with photosynthesis. The process of rubber biosynthesis requires three components: acetyl-CoA as building block, NADPH as reducing agent, and ATP as energy source. All three components are generated by degradation of carbohydrate which is an end-product of photosynthesis[3]. The carbohydrates are being synthesized and accumulated throughout the photosynthesis period, increasing in level by the end of the day[13,14]. During the dark period one or more of the three components become a limiting factor causing the decline in HMG-CoA reductase activity. The requirement of these components for rubber biosynthesis could be considered analogous to the study on diet fed to animals. The diurnal variation in *Hevea* HMG-CoA reductase is probably a natural daily cycle of fasting and feeding similar to that suggested earlier for the rat liver enzyme[8,15].

It is generally observed that rubber trees tapped after sunrise give less latex than those tapped during night time[16]. Supporting data were obtained in this study. The percentage of rubber content in latex in either clone RRIM 600 or KRS 21 was highest at sunset, 18.00 h, and remained relatively high throughout the night. During the daylight period the rubber content was lower, and it was lowest at 10.00 (fig. 2). The percentage of rubber content varied diurnally, closely parallel to the pattern exhibited by HMG-CoA reductase activity. This relationship showed that the level of HMG-CoA reductase activities was closely associated with the rubber content in the latex. The correlation suggested the importance of this enzyme in the regulation of rubber biosynthesis.

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High resolution isoelectric focusing of juvenile hormone esterase activity from the hemolymph of *Trichoplusia ni* (Hübner)

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Summary. Juvenile hormone esterase (JHE) activity from the hemolymph of larval *Trichoplusia ni* was analyzed by two different isoelectric focusing (IEF) methodologies. Use of techniques capable of progressively higher resolution split ultimately what appeared at lower resolution to be a single peak into two discrete peaks of JHE activity (pI 5.5 and 5.3). Neither peak was a degradation artifact of the other caused by conditions of IEF.
Key words. Juvenile hormone esterase; *Trichoplusia ni;* isoelectric focusing.

Juvenile hormone (JH), an endocrine of central importance in insect metamorphosis, is thought to be regulated, in part, by ester hydrolysis. JH esterase (JHE) activity is present in many insect orders[2], and has been shown to peak in the hemolymph twice during the last larval stadium of many Lepidoptera[3]. Recent extensive studies and reviews have centered around *Trichoplusia ni* (Hübner) as a model for the study of lepidopteran JHE[4,5]. Their results have led to the conclusion that there are not multiple forms of JHE in the *T. ni* model system, rather JH ester hydrolysis is due to a single enzyme or polypeptide chain.

In the present study we have re-examined the results of isoelectric focusing techniques with respect to the number of *T. ni* JH esterases which are identifiable on polyacrylamide IEF gels.

Materials and Methods. Insects. The laboratory colony of *T. ni* was maintained at 28 °C, 14:10 light:dark[6]. Last day, feeding stage larvae were bled as described previously[3]. Chemicals. Radiolabeled JH I, II and III were obtained from NEN while unlabeled homologs were obtained from Sigma or Calbiochem-Behring. Preliminary tests showed profiles of JHE activity obtained from IEF gels are the same using all three homologs.

Isoelectric focusing. Polyacrylamide gels (5% with 3% cross linking) were cast and held overnight prior to use. LKB Ampholine® or Pharmacia Pharmalyte® was used to create pH gradients of either 3.5–9.5 or 4–6.5. Alternatively, LKB Immobiline® compounds (which are not as salt sensitive) were used to create a pH gradient of 5–6. The gels were run on a LKB Multiphor apparatus, in either the 9-cm or 23-cm direction. 20 µl of hemolymph diluted 1:1 with double distilled water (dHOH) were added to an 0.5 × 1.0 cm paper wick placed at the edge of a lane. On some Immobiline gels 60 µl of such diluted hemolymph were added in 3 portions. After electrofocusing at power settings and a time duration described by the supplier (see figure legend), the gel was sliced into sections. Each section was placed into 400 µl of dHOH, for pH determination or phosphate buffer (pH 7.4, I = 0.2 M) for assay of enzyme activity, and eluted overnight at 4 °C. Each sample of eluate was appropriately diluted and assayed in duplicate for JHE activity.

JHE assay. A partition assay previously described[7] was used to measure JHE activity, except that samples in which the enzymatic activity was terminated with the methanolic stopping solu-

tion prior to the addition of substrate were used as control blanks.

Results. Wide range ampholine gels. Wide range gels run across a 9-cm gradient of pH 3.5–9.5 consistently produced one peak of JHE activity (fig. a). The average pI of the JHE activity was near 5.3. Since these gels were sliced into 0.5-cm sections, their limit of resolution is 0.33 pH units.

Narrow range Ampholine or Pharmalyte gels. When narrow range gels (pH 4–6.5) were run across 9-cm, a single peak of JHE activity was obtained (fig. b). However, sometimes a split peak occurred. The average pI of the single or major peak was 5.4. Since these gels were sliced into 0.5-cm sections, their limit of resolution is 0.14 pH units.

In order to determine whether the occasional split peak was due to 2 closely focusing proteins or merely to an aberration in the pH gradient, similar gels were run, except that they were sliced into 0.25-cm sections to increase the resolution to 0.07 pH units. Using this methodology, 2 closely focusing but clearly separated peaks were observed (fig. c). The pI of the large peak was approximately 5.5, while that for the smaller, more acidic peak was near 5.3.

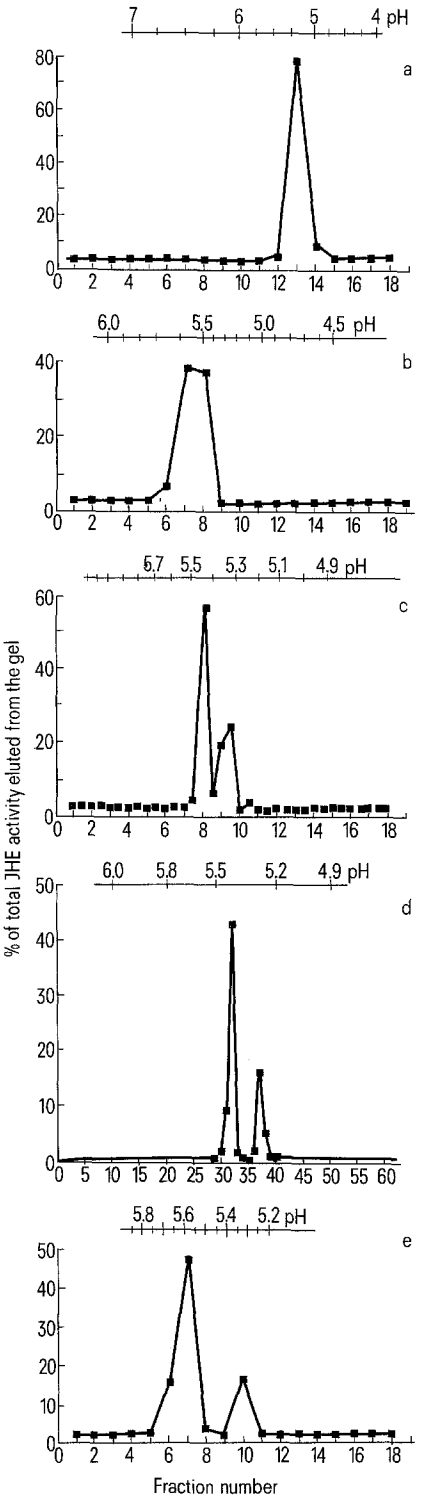
Although two peaks were indicated, convincing data required that we obtain separation by at least several gel sections. Thus, gels of pH 4–6.5 were run across 23-cm and sliced into 0.25-cm sections, providing a resolution of 0.03 pH units. Two distinct peaks were again obtained, separated by at least three gel fractions (fig. d). The average pI for the major peak was 5.5, and that for the smaller, more acidic peak was 5.3.

Very narrow range Immobiline gels. Ampholine gels have been occasionally observed by some researchers to artifactually cause some single proteins to appear in multiple forms on IEF due to the binding of the ampholytes to the protein. As a control, a second methodology was employed. Immobiline gels were used, in which the functional groups establishing the pH gradient are covalently bound to the gel and are thus unable to bind to and migrate with the protein. Using a pH gradient of 5–6 we again obtained two clearly separate peaks of JHE activity (fig. e). The average pI for the larger peak, as estimated by marker proteins, was 5.6 and that for the smaller acidic peak was 5.3. Since these gels were run across 9-cm and sliced into 0.5-cm sections, their resolution is 0.06 pH units.

Extraction and re-electrofocusing. After narrow range Ampholine IEF each peak was separately eluted, concentrated by ultracentrifugation, and reelectrofocused on a narrow range Ampholine gel. The JHE peak with a pI of 5.5 refocused as a single peak with pI again near 5.5. The JHE peak with a pI of 5.3 refocused as a single peak with pI again near 5.3. Thus, each peak of JHE activity is real and not an artifact of the other caused by exposure of IEF conditions.

Discussion. Trichoplusia ni is currently the most popular model insect for studies on the regulation and biochemical properties of JHE[8–13]. The present study has important implications for the previous interpretation of IEF data used to construct a model of *T. ni* JHE. That interpretation has led to a model in which a single protein accounts for most if not all of the hemolymph JHE activity.

The data from the present study show that narrow range (pH 4–6.5) IEF gels run across 9-cm and sliced into 0.5-cm sections are not sufficient to consistently and clearly distinguish the 2 separate peaks of JHE activity, although a split peak is rarely observed. One group cut smaller fractions and observed 2 peaks of pI 5.3 and 5.5. Unfortunately, those authors disregarded the activity focusing at 5.3 by speculating it to be an artifact or degradation product. The conclusion that the pI 5.3 activity was artifactual apparently influenced subsequent researchers[10] who cast and ran 23-cm gels in a manner normal for 9-cm gels. Although they observed 2 incompletely separated peaks with pIs of 5.5 and 5.3, the smaller acidic peak was discounted. However, we have found that incomplete peak separation results unless the Ampholine or Pharmalyte concentration is increased propor-



JH esterase activity on isoelectric focusing gels. *a* Wide range Ampholine gel (pH 3.5–9.5), sliced into 0.5-cm sections for JHE assay, run 1 h × 25 W. *b* Narrow range Ampholine gel (pH 4–6.5), run across 9-cm sliced into 0.5-cm sections, run 3 h × 25 W. *c* Same as b except sliced into 0.25-cm sections, run 3 h × 25 W. *d* Same as e, except run across 23-cm, run 12 h × 50 W. *e* Very narrow range Immobiline gel (pH 5–6), run across 9-cm and sliced into 0.5-cm sections, run 16 h × 25 W. All wattage settings indicate upper power limit of constant power mode.

tionally to the increase in distance and the running time similarly increased. Neither peak of JHE activity appears to be an artifact of electrofocusing media or conditions. It is possible that in the purification studies on *T. ni.* which obtained a single IEF peak[10,14] conditions used were not of sufficient resolution or one of the two JHE activities was lost during purification.

The results presented here indicate that previous IEF data used to construct and promote the popular *T. ni,* single protein model of JHE[7,8,15] should be reevaluated. Since the careful application of IEF methodology actually shows the presence of at least 2 discrete JHE activities, studies of JHE purification, structure, inhibition[16,17] and regulation should first carefully determine the nature of the JHE activity being studied.

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Glycine absorption from the small intestines of rats after secondary infections with *Eimeria nieschulzi*

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Summary. A second (challenge) infection of *Eimeria nieschulzi* in clinically immune rats did not produce weight gain depression but caused a decrease in the absorption of glycine from the ileum. The malabsorption due to challenge was equivalent to that caused by the primary infection which did cause weight loss.
Key words. Eimeria nieschulzi; coccidia; challenge infection; malabsorption; ileum; glycine.

As a result of epithelial damage, intestinal coccidiosis causes malabsorption of nutrients including proteins and amino acids in chickens[1,2] and in rats[3,4]. Absorption has usually been examined during the acute phase of the disease and to our knowledge, apart from a recent report[5] using *Eimeria acervulina* in chickens there has been no study of absorption resulting from challenge infections of immune animals. The present study compares the absorption of glycine from the ilea of rats during the acute and recovery phases of primary *E. nieschulzi* infections and secondary/challenge infections.

Male Wistar rats (120–150 g) were orally inoculated with sporulated oocysts of *E. nieschulzi* originally supplied by Dr Dawn Owen. In expt. 1 the primary and secondary infections were 10^5 oocysts and in expt. 2, the primary infection was 5000 oocysts and the second (challenge) infection was 5×10^6 oocysts. The oocyst dose in expt. 1 was chosen because it was known that the resulting infection caused malabsorption[3], and in expt. 2 doses of oocysts of the order of 5000 produce a good immune response[6]. Uninfected control groups provided the comparison for normal weight gains and the normal data on absorption, animals being sacrified at intervals throughout the experimental period. Control values for absorption remained constant throughout the experimental period and are represented in the figures under day 0. Glycine absorption through the ileum was measured in vitro by using a modified everted sac method[7] as described elsewhere[8] since it is believed to be a method particularly sensitive to damage of the epithelial layer. Pairs of rats were selected at random over the experimental period for absorption measurements conducted on 4–6 ileal sacs per animal. Pieces of ileum from each rat were processed for examination by light and scanning electron microscopy. The criterion of immunity to reinfection was taken to be the maintenance of normal weight gains linked to the absence of clinical signs.

In expt. 1, the amount of serosal glycine fell significantly during the primary infection (fig. 1) the lowest amount being recorded on day 7 post infection (p.i.) that is one day before the lowest weight gain depression. The weight gains shown are typical for this species of *Eimeria*. From day 8 p.i. the malabsorption decreased and although there was not a return to normal values during the recovery period there was no significant difference between the control value and those at day 21 p.i. when the second inoculation was administered. A similar pattern of depressed absorption followed the second infection (fig. 1) although there were no clinical signs of infection as shown by the maintenance of good growth. There was no obvious evidence by light microscopy of villous damage on day 21 p.i. or 5–8 days after the second infection but some flattening of villi was seen by scanning electron microscopy. It is of interest to note that 16 days after both the primary and secondary inoculations there was a further decrease in absorption. There was no evidence that this was caused by self-infection, and there is no satisfactory explanation at present. This double decrease in absorption did not occur in expt. 2 (fig. 2). In this experiment the weight gains were similar to those in expt. 1 and the absorption of glycine