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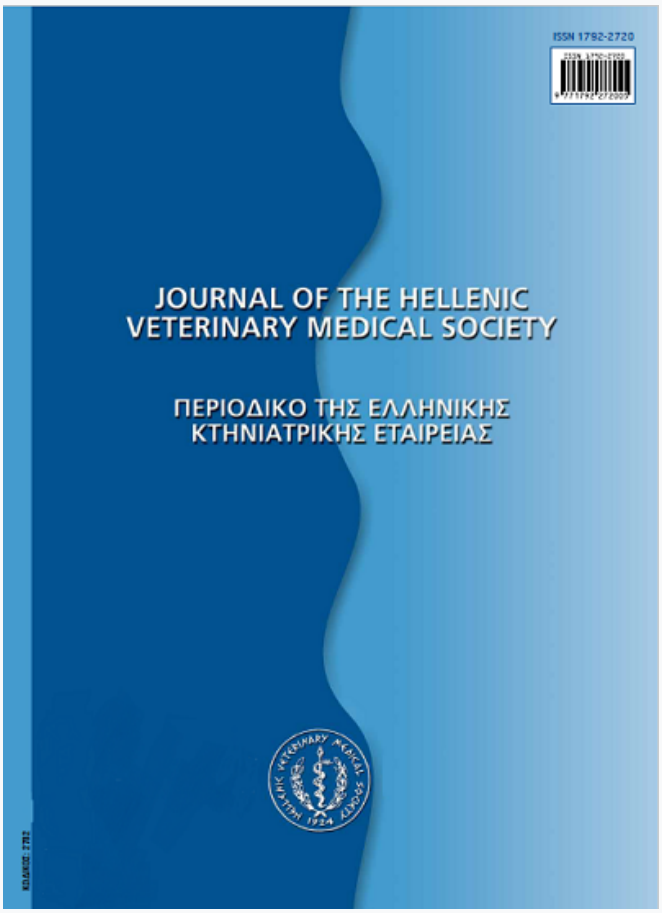

Acute phase response in experimentally infected broilers with avian infectious bronchitis virus serotype 4/91

S. SEIFI, S. H. ALIAN SAMAKKHAH, K. ABSALAN FARD

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Research article
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Acute phase response in experimentally infected broilers with avian infectious bronchitis virus serotype 4/91

Seifi S.[1], Alian Samakkhah S.H.[2], Absalan Fard K.[2]

[1]*Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran*

[2]*Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

ABSTRACT. Measurement of acute phase proteins is important for diagnosis of animal diseases. In the present study, effects of avian infectious bronchitis virus infection on acute phase response and acute phase proteins were determined. Thirty one-day-old commercial broiler chicks were reared in experimental facilities and, at the age of 21 days, were challenged intranasally with 0.2 mL of allantoic fluid virus suspension (titre $10^{6.5}$ EID50 per 0.1 mL). Serum samples were obtained prior to challenge and on days 1, 2, and 5 post-inoculation. Haptoglobin, serum amyloid A and C- reactive protein concentrations were measured. Our results showed that all investigated acute phase proteins increased significantly after infection, with mean maximum concentrations between 24 h and 48 h. No correlation was observed between plasma acute phase proteins in the chickens prior and post inoculation of the virus. Haptoglobin was most sensitive factor to change in the exposed birds.

Keywords: acute phase proteins, chicks, infectious bronchitis, 4/91

Correspondence: S. Seifi,
Faculty of Veterinary Medicine,
Amol University of Special Modern Technologies, Amol, Iran.
E-mail: saeedseifi@umz.ac.ir

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INTRODUCTION

Acute phase proteins (APPs) constitute a group of proteins, the blood concentrations of which change rapidly in response to stress such as infection and tissue damage. C-reactive protein (CRP), haptoglobin (Hp), ceruloplasmin (Cp), serum amyloid A (SAA), α-1 acid glycoprotein and fibrinogen are positive APPs, and their blood concentrations are elevated mainly due to increased production in the liver (Canova et al., 1999). On the other hand, albumin and transferin are known as negative APPs (Ceron et al., 2005). The synthesis and release of plasma APPs from the liver is regulated by inflammatory mediators. These mediators fall into four major categories: interleukin-6-type cytokines, interleukin-1-type cytokines, glucocorticoids and growth factors. Cytokines mainly stimulate the APP gene-expression, while glucocorticoids and growth factors function more as modulators of cytokine action (Baumann and Gauldie, 1994). The concentration of these proteins is generally low to non-detectable in healthy animals and elevations are used to diagnose and monitor inflammatory diseases (Feldman et al., 2000; Jiang et al., 2010). It has been suggested that measuring plasma levels of these proteins could be useful for monitoring poultry health (Tohjo et al., 1995; Asasi et al., 2013) and for identifying inaccessible lesions at meat inspection (Saini and Webert, 1991).

Infectious bronchitis virus (IBV) is a major cause of economic losses in poultry and can be involved in respiratory disease, nephritis and poor egg production and quality (Cavanagh, 2007). However, these signs are not specific to IBV. Therefore, diagnostic tools are needed to identify IBV infections in relation to a clinical problem in the field. Serotype 4/91 of IBV (also named 793/B and CR88) is one of the most common IBV serotypes throughout the world. It has spread to many parts of the world, including Russia and the Middle and Far East (Jackwood, 2012). In this study, we evaluated and compared concentrations of three APPs in chicks experimentally infected with IBV serotype 4/91 for use as potential diagnostic tools of the infection.

MATERIALS AND METHODS

Virus isolate

Serotype 4/91 IBV was isolated from broiler flocks in Iran (Seifi et al., 2010). The isolate was preserved in allantoic fluid at -70 °C. The preserved agent was thawed and then propagated by inoculation (0.1mL) into the allantoic cavity of nine-day-old chicken embryonated eggs.

Eggs were obtained from a respiratory disease-free flock. The eggs were incubated at 37 °C for 72 hours before being stored at 4 °C for 12 to 18 hours. Then the allantoic fluid was collected and frozen at -70°C for stock solution. The embryo infectious dose 50% (EID50/0.1ml) was calculated according to the Reed and Muench formula (1938).

Experimental design

Thirty one-day-old commercial broiler chicks (Ross breed) were reared in experimental facilities. Feed and water were supplied *ad libitum*. No vaccine was administered to the chicken used in our experiment. Prior to virus challenge, all birds were examined for antibodies against IBV (Flock Check IBV ELISA test kit; IDEXX Laboratories Inc., Westbrook, ME, USA) and were found seronegative. At the age of 21 days, blood samples were collected from all chicks (as control group). Blood samples were taken from the wing vein, collected in tubes with and without EDTA. After bleeding, all birds were challenged intranasally with 0.2 mL of allantoic fluid virus suspension (titre $10^{6.5}$ EID50 per 0.1mL). The chickens were monitored daily for clinical signs and mortality after challenge. Five birds were bled at 1, 2, and 5 day post-inoculation as described above. The sera were separated by centrifugation at 3000 rpm for 10 minutes and were numbered and kept frozen (-20 °C) till the day of analysis. On day 3 post-inoculation, three chickens were randomly selected and euthanized and gross lesions were recorded.

Biochemical measurements

CRP was measured by a sandwich ELISA kit (Uscn Life Science, Inc., Wuhan, China) according to the manufacturer's instructions. The analytical sensitivity of the test in serum was considered less than 0.211 ng/mL based on the manufacturer's data sheet. Hp was measured according to prevention of the peroxidase activity of haemoglobin, which is

directly proportional to the amount of Hp. The analytical sensitivity of this test in serum has been determined as 0.0156 mg/mL for Hp by the manufacturer (Tridelta Development Plc, Wicklow, Ireland). SAA was measured by a solid phase sandwich ELISA. The analytical sensitivity of this test in serum has been determined as 0.3 μg/mL for SAA by the manufacturer (Tridelta Development Plc, Wicklow, Ireland).

Statistical analysis

All results were expressed as means ± standard error (se). The obtained data were subjected to the Kolmogorov-Smirnov test for normality. The parametric repeated measures test was used to compare observations repeated on the same subjects. Comparisons between infected and control at each time point were assessed using the Bonferroni test. Pearson correlation coefficients were calculated to determine relationships between variables. To evaluate which factor was more sensitive to change in diseased birds compared to healthy control birds, receiver operating characteristics (ROC) analysis was done and area under the curve (AUC) were compared. For all analyses, $P<0.05$ was considered as statistically significant. All calculations were performed with SPSS (version 20) and Medcalc softwares.

RESULTS

Clinical signs and Gross findings

Half (15/30) of the chickens showed mild tracheal rales, coughing and gasping 24 hours post-inoculation. These symptoms disappeared 4 days later. No mortality was observed during the experiment. In the necropsy, slight hyperaemia and oedema in tracheal mucosa and pale and swollen kidneys were observed.

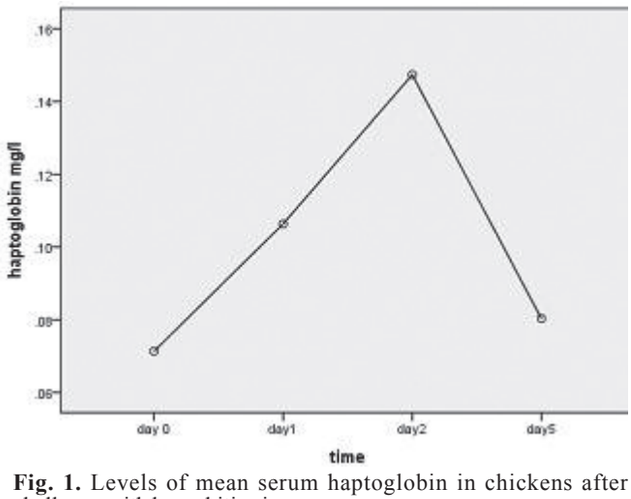

Fig. 1. Levels of mean serum haptoglobin in chickens after challenge with bronchitis virus.

Acute phase proteins

The plasma APPs (haptoglobin, serum amyloid A and C-reactive protein) concentrations observed in exposed birds and in control (prior to inoculation of bronchitis virus) are presented in Table 1.

Haptoglobin

Repeated measures ANOVA with a sphericity assumed correction determined that mean Hp concentration differed statistically significantly between time points ($F(3, 87) = 82.453$, $P< 0.001$). Post hoc tests using the Bonferroni correction revealed that prior of inoculation individual levels of Hp were found to be 0.07±0.004 (95% CI: 0.062-0.08) mg/L and post inoculation of bronchitis virus, the significant changes in the concentration of Hp were observed during study (Table 1). The highest mean concentrations of Hp were observed at day 2 post-

Table 1. Plasma APPs (haptoglobin, serum amyloid A and C-reactive protein) concentrations observed in 30 exposed birds, 24h, 48 h, and 120 h after the inoculation of avian infectious bronchitis virus; results are expressed as mean±standard error of the mean.

	Prior to inoculation of bronchitis virus	Post-inoculation of bronchitis virus		
	Day 0 (control)	Day 1	Day 2	**Day 5**
Haptoglobin (mg/L)	0.071±.004[a]	0.106±.003[b]	0.147±.004[c]	0.080±.003[a]
Serum amyloid A (μg/mL)	1.275±.018[a]	2.231±.034[b]	2.852±.021[c]	1.440±.020[d]
C-reactive protein (μg/mL)	1.3±0.014[a]	1.65±0.011[b]	1.77±0.014[c]	1.4±0.007[d]

inoculation and were 0.147±0.004 (95% CI: 0.139-0.156) mg/L. The mean concentration of Hp had increased by 24 h after inoculation and from that time-point were significantly higher as compared to control (pre-inoculation) (P<0.001). Hp concentration by 120 h after inoculation was not significant as compared to control (prior to inoculation) (P>0.05, P=0.923). Figure 1 shows that most birds have haptoglobin concentration ranging from 0.06 to 0.16 and the highest mean concentrations of Hp were observed at day 2 post inoculation.

Serum amyloid A

Repeated measures ANOVA with a sphericity assumed correction determined that mean SAA concentration differed statistically significantly between time points (F (3, 87) = 902.006, P< 0.001). Significant increase of SAA after infection, compared to controls (prior to inoculation), was observed only at 24 h, 48 h and 120 h after inoculation of bronchitis virus (P<0.001). Prior of inoculation individual levels of SAA were found to be 1.27±0.018 (95%CI: 1.24-1.31) μg/mL; post-inoculation of bronchitis virus, significant changes in the concentration of SAA were observed during study (Table 1). This plot (Figure 2) shows that most birds have SAA concentration ranging from 1 to 3 and the highest mean concentrations of SAA were observed at day 2 post-inoculation.

C-reactive protein

Repeated measures ANOVA with a sphericity assumed correction determined that mean CRP concentration differed statistically significantly between time points (F (3, 87) = 338.47, P< 0.001). Prior to inoculation, experimental chickens had CRP serum concentration 1.3±0.014 μg/mL. Twenty four hour after infection the mean concentration of CRP reached 1.65 ± 0.011 μg/mL. Significant difference, compared to controls (prior to inoculation), were seen 24 h, 48 h and 120 h (P<0.001). The maximum mean level was observed at day 2 (48 h after inoculation) and reached 1.77 ± 0.014 μg/mL. This plot (Figure 3) shows that most birds have C-reactive protein concentration ranging from 1.2 to 1.8 and the highest mean concentrations of CRP were observed at day 2 post inoculation.

DISCUSSION

Acute phase proteins are blood proteins primarily synthesized by hepatocytes as part of the acute phase response (APR). The APR is part of the early-defence or innate immune system, which is triggered by different stimuli including trauma, infection, stress, neoplasia, and inflammation (Cray et al., 2009).

Tissue damage include tracheitis, bronchitis, inflammation of the lungs and thickened and frequently cloudy air sacs indicates a widespread inflammatory reaction in IBV infected chicks thus causes release and elevation of APPs. According to the results, all investigated APPs (haptoglobin, serum amyloid A and C-reactive protein) increased significantly after infection with infectious bronchitis virus, with mean maximum concentration from 24 h to 48 h. No correlation was observed between plasma APPs in the chickens prior and post inoculation of bronchitis virus. Results for ROC analysis showed that area under curve for Hp (Days1, 2, 5), SAA (Days1, 2, 5) and CRP (Days1, 2, 5) were 0.90, 0.82 and 0.80, respectively. According to AUC, Hp was most sensitive factor to change in the exposed birds. However, there was no significant difference for AUC between plasma APPs in different days.

Nazifi et al. (2010; 2011) showed a significant increase in SAA and Hp levels in chicks infected with Gumboro and IB viruses which is in line with results of the present study. Our results indicate that both Hp and SAA are detectable in the serum 24 h after virus inoculation and their levels changes

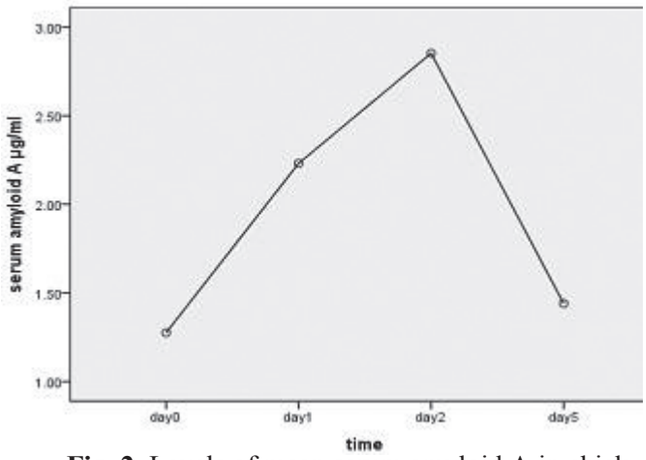

Fig. 2. Levels of mean serum amyloid A in chickens after challenge with bronchitis virus.

indicate that they are more sensitive indicators than CRP. Mosleh et al. (2013) showed that elevation in the serum Hp was observed from 24 h hour after inoculation of avian influenza virus in chukar partridge, while the concentrations of SAA, TNF-α and IFN-γ peaked at 3 days post inocoulation. There are substantial differences between species in the relative changes in acute phase protein production following stimulation. Thus, while CRP is a major APP in humans, dogs and swine, in ruminants its serum concentration is hardly altered by the presence of infection or inflammation. In contrast, Hp is a major APP in ruminants in which species it has a negligible circulating level in healthy animals, but increases over 100 fold on stimulation. In contrast in dogs, Hp is a constitutive serum protein and moderate APP (Conner et al., 1988; Eckersall et al., 1996). There is little information about APPs in birds. In chickens only a few APPs have been described so far. Of these the Plasma α1-Acid Glycoprotein (AGP), Serum amyloid A (SAA), Transferrin, and Ovotransferrin (Inoue et al., 1997; Chamanza et al., 1999; Holt and Gast, 2002; Xie et al., 2002), can be mentioned. Nielsen et al. (1999), described concentration of serum MBL increased about twofold in 3-7 days after infection with infectious bronchitis virus. According to the AUC, concentration of serum Hp was the most sensitive factor to change in the experimentally infected birds. Thus, increase in serum Hp concentration may be a good indicator of inflammatory process associated with IBV in chicks. The highest mean concentrations of Hp, SAA, and CRP were observed at day 2 post inoculation, which showed that sampling in early days after virus challenge is useful in diagnosis.

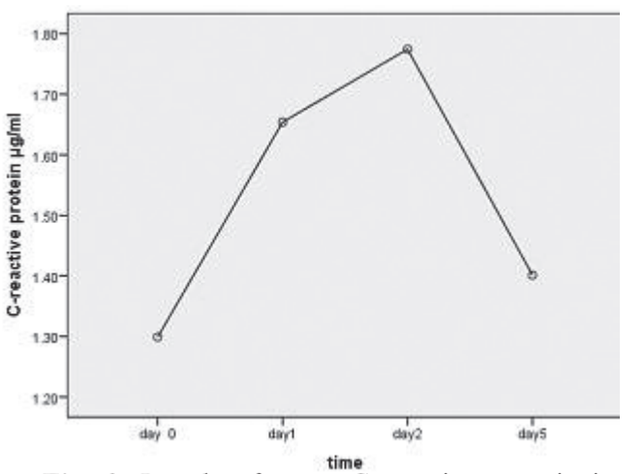

Fig. 3. Levels of mean C-reactive protein in chickens after challenge with bronchitis virus.

In general, IBV infections can be diagnosed by detection of IBV virus or the specific antibody response. The most common assays for routine use of virus detection are virus isolation (VI) and polymerase chain reaction (PCR), and for antibody detection is enzyme linked immunosorbent assay (ELISA). The level of success in detection of IBV after a disease outbreak is influenced by many factors of which the time between onset of infection and sampling, the level of immunity in the chicken at the moment of infection, and the number, choice and quality of sampled organs are the most important. Some of these methods such as virus isolation (VI) can be laborious, time-consuming and costly. The sensitivity of the RT-PCR is usually low when performed directly on organs. On the other hand, interpretation of serological results can be complicated by a number of factors including presence of immunity at time of vaccination/infection, cross-reactions between serotypes, and occurrence of new or unexpected IBV strains. Antibodies can first be detected by ELISA within a week after vaccination or infection. Because of the short period between infection and the detection of the first antibodies by ELISA, the first of paired sampling must be done at the first signs of IBV. If the first sampling is not done in time, seroconversion can be missed (De Wit, 2000).

It is hypothesized that the measurement of acute phase proteins could be a way to quickly identify disease. Our results showed APPs increased significantly after infection with IBV, with mean maximum concentration between 24 h and 48 h, and Hp was most sensitive factor to change in the exposed birds.

CONCLUDING REMARKS

In conclusion, IBV leads to tissue damages and inflammatory effects, so stimulates the synthesis of APPs. Significant changes in CRP, SAA and Hp concentration were observed in experimentally infected chicks, but Hp had the most obvious change so it is the most sensitive index.

CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

REFERENCES

Asasi K, Mohammadi A, Boroomand Z, Hosseinian SA, Nazifi S (2013) Changes of several acute phase factors in broiler chickens in response to infectious bronchitis virus infection. Poult Sci 92:1989-1996.

Baumann H, Gauldie J (1994) The acute phase response. Immunol Today 15:74-80.

Canova CR, Courtin C, Reinhart WH (1999) C-reactive protein (CRP) in cerebro-vascular events. Atherosclerosis 147:49-53.

Cavanagh D (2007) Coronavirus avian infectious bronchitis virus. Vet Res 38:281–297.

Ceron JJ, Eckersall PD, Martynez-Subiela S (2005) Acute phase proteins in dogs and cats: current knowledge and future perspectives. Vet Clin Pathol 34:85-99.

Chamanza R, Tuossaint MJ, van Ederen AM, van Veen L, Hulskamp-Koch C, Fabri TH (1999) Serum amyloid A and transferring in chicken. A preliminary investigation of using acute-phase variables to assess diseases in chickens. Vet Q 21:158-162.

Cray C, Zaias J, Altman NH (2009) Acute phase response in animals: a review. Comp Med 59:517-526.

Conner JG, Eckersall PD, Ferguson J, Douglas TA (1988) The acute phase response in the dog following surgical trauma. Res Vet Sci 45:107-110.

De Wit JJ (2000) Detection of infectious bronchitis virus. Avian Pathol 29:71–93.

Eckersall PD, Saini PK, Mccomb C (1996) The acute-phase response of acid-soluble glycoprotein, alpha(1)-Acid glycoprotein, ceruloplasmin, haptoglobin and c-reactive protein, in the pig. Vet Immunol Immunopathol 51:377-385.

Feldman BV, Zinkl JG, Jain NC (2000) Schalm's Veterinary Hematology, 5th edn, Williams & Wilkins, Philadelphia, 1152 pp.

Holt PS, Gast RK (2002) Comparison of the effects of infection with Salmonella enteritidis, in combination with an induced molt, on serum levels of the acute phase protein α1-acid glycoprotein, in hens. Poult Sci 81:1295-1300.

Inoue M, Satoh W, Murakami H (1997) Plasma α1-acid glycoprotein in chickens infected with infectious bursal disease virus. Avian Dis 41:164-170.

Jackwood MW (2012) Review of infectious bronchitis virus around the world. Avian Dis 56:634-641.

Jiang Z, Schatzmayr G, Mohnl M, Applegate TJ (2010) Net effect of an acute phase response–Partial alleviation with probiotic supplementation. Poult Sci 89:28-33.

Mosleh N, Nazifi S, Nili H, Habibi H (2013) Effect of H9N2 virus infection on the acute phase response in Chukar Partridges (Alectoris Chukar). Bulgarian J Vet Med 16:20-28.

Nazifi S, Dadras H, Hoseinian SA, Ansari-Lari M, Masoudian M (2010) Measuring acute phase proteins (haptoglobin, ceruloplasmin, serum amyloid A, and fibrinogen) in healthy and infectious bursal disease virus-infected chicks. Comp Clin Pathol 19:283-286.

Nazifi S, Tabande MR, Hosseinian SA, Ansari-Lari M, Safari H (2011) Evaluation of sialic acid and acute-phase proteins (haptoglobin and serum amyloids A) in healthy and avian infection bronchitis virus virus-infected chicks. Comp Clin Pathol 20:69-73.

Nielsen OL, Jensenius JC, Jorgensen PH, Laursen SB (1999) Serum level of chicken mannan-binding lectin (MBL) during virus infections, indication that chicken MBL is an acute phase reactant. Vet Immunol Immunopathol 70:309-316.

Reed LJ, Muench H (1938): A simple method of estimating fifty per cent endpoints. Am J Epidemiol 27:493-497.

Saini PK, Webert DW (1991) Application of acute phase reactants during antemortem and postmortem meat inspection. J Am Vet Med Assoc 198:1898-1902.

Seifi S, Asasi K, Mohammadi A (2010) Natural co-infection caused by avian influenza H9 subtype and infectious bronchitis viruses in broiler chicken farms. Vet Arhiv 80:269–281.

Tohjo H, Miyoshi F, Uchida E, Niyama M, Syuto B, Moritsu Y, Ichikawa S, Takeuchi M. (1995) Polyacrylamide gel electrophoretic patterns of chicken serum in acute inflammation induced by intramuscular injection of turpentine. Poult Sci 74:648655.

Xie H, Huff GR, Balog JM, Holt P, Rath NC (2002) Identification of ovotransferrin as an acute phase protein in chickens. Poult Sci 81:112-120.