BIOCHEMICAL CHANGES IN CHICKEN SERUM DURING INFECTION WITH STRAINS OF NEWCASTLE DISEASE VIRUS OF DIFFERING VIRULENCE. I. ENZYME STUDY

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SUMMARY

Chickens aged 5-6 weeks were inoculated with 3 strains of Newcastle disease virus of differing pathogenicity. The levels of the enzymes lactate dehydrogenase (LDH), isocitrate dehydrogenase (ICDH), cholinesterase (ChE), alkaline and acid phosphatase (ALP and AcP), glutamate-oxaloacetate and glutamate-pyruvate transaminases (GOT and GPT) in serum were measured. Significant decreases in the levels of ChE and ALP were found in chickens infected with high doses of a velogenic strain of virus. The levels of LDH, ICDH and GOT were elevated in these chickens, but the levels of AcP and GPT were unchanged. ALP levels were slightly decreased and LDH levels were slightly elevated in chickens that were inoculated with a mesogenic strain. No significant changes in enzyme levels were found in chickens infected with lentogenic virus. Changes in enzyme levels were found to be correlated with the clinical findings in the infected chicks.

INTRODUCTION

The measurement of changes in levels of serum enzymes has become a well established technique for detecting and identifying infectious diseases (Freedland and Kramer, 1970). The disease may lead to tissue damage and release of cell enzymes into the blood so that the enzyme profile of the injured tissues is reflected by a corresponding rise in the levels of the same enzymes in the serum.

Very little is known about serum enzyme changes in fowls during viral infections. Changes have been described in the levels of phosphatases, particularly alkaline phosphatase, following infection (Lesher and Burmester, 1959; Cornelius et al., 1959; Bide, 1972).

The biochemical events in the course of infection of chickens with virus (NDV) have been studied to a limited extent. Akün and Schulte (1953) reported an increase in ALP levels in blood and cerebrospinal fluid. Pasley and Auer (1958) showed an elevation in AcP levels in the pituitary without corresponding changes in the level of ALP.
More recently, Lust and Squibb (1967) described a sharp decrease in ALP levels in chicken serum following a decrease of enzyme level in the gut and an elevation in the liver.

This work describes investigations in which changes in serum enzyme levels in chickens inoculated with NDV were studied. An attempt was made to find a correlation between the biochemical changes and virulence of different strains of the virus.

MATERIALS AND METHODS

Chickens
Four hundred one-day-old male Leghorn x New Hampshire chicks were allocated into groups of 60, each group was kept in a separate room. Inoculation with virus was carried out when the birds were 5-6 weeks old. Conditions of husbandry were similar, before and during the experiments.

Virus
Three strains of NDV of differing virulence were used: La Sota, a lentogenic strain that is used for vaccination of newly hatched chicks; Vineland-Israel Lot No.3089, a mesogenic strain used to vaccinate older chickens, and NDV-Israel 1967, a velogenic strain isolated in Israel in 1967.

Procedure
Chicks were inoculated intramuscularly with $10^{8.0}$ ELD$_{50}$ of virus. Blood samples (4ml) were drawn from the heart immediately before injection and at intervals thereafter. Five birds from each group were bled each time. Blood was not taken from the same chick more than once. After coagulation of the blood, sera were collected and assayed immediately for enzyme activities.

Enzyme assay
Lactate dehydrogenase (LDH) activity was measured spectrophotometrically according to the method of Bergmeyer (1963), with the modification that 0.01ml serum was used in a final volume of 1ml. Isocitrate dehydrogenase (ICDH) activity was determined spectrophotometrically according to the method of Wolfson et al. (1957). Cholinesterase (ChE) activity was determined by a colorimetric method using 5,5'-dithiobis-(2-nitro-benzoate) as an indicator which is reduced by the thiocholine liberated to form a yellow compound. 3ml of 0.05 phosphate buffer at pH 7.2, 0.02ml serum and 0.1ml of 0.156 M S-acetylthiocholine iodide solution were pipetted into a glass cavette. Optical density readings were taken at 405nm for 5 minutes. The activity was calculated from the formula $E_{405 \text{nm}}/30 \text{sec} \times 23400 = \text{milliunits/ml of serum}$.

Alkaline phosphatase and acid phosphatase activities were determined colorimetrically by measuring the colour of p-nitrophenol liberated from its phosphate compound (Bessey et al., 1946). Glutamate-oxalacetate transaminase and glutamate-pyruvate transaminase activities were measured colorimetrically according to the method of Reitman and Frankel (1957).

The assays of these enzymes were carried out using Pye Unicam SP 800 A and SP 1800 spectrophotometers.

A unit of enzyme is defined as the amount of enzyme which converts 1µ mole of substrate per minute at the reaction conditions described.

Infectivity
Sera were diluted with phosphate buffered saline (PBS) 1:2, 1:10, 1:100, and 0.2ml
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of each dilution was injected into the allantoic sac of 10-day-old embryonated eggs. The LD50 of the serum was determined according to the method of Reed and Muench (1938).

RESULTS

Inoculation of chicks with the virulent strain NDV-Israel 1967 resulted in definite enzyme changes (Text-figs.1 and 2). When the chicks were inoculated with 10^8.0 ELD50 of virus, there was a decrease of 33% (P<0.001) in the levels of cholinesterase at 48 hours post-inoculation (Text-fig.1). The greatest rate of change, about 42% (0.02 > P > 0.01) was observed 72 hours after infection, at the terminal stage of the disease. An elevation of 50% (0.05 > P > 0.02) in the level of LDH was found 24 hours post-inoculation and this rose further to more than 200% (0.01 > P > 0.001) 72 hours after infection. ICDH levels were also elevated 48 and 72 hours after inoculation by 70 and 350% respectively (P<0.001) (Text-fig.1).

Text-fig.1. Effect of infection of chickens with NDV-Israel 1967 on serum LDH, ChE and ICDH activities. Closed circles – infected chickens. Open circles – uninfected chickens. Mean enzyme activities ± standard error of the mean (± SEM) expressed as milliunits per ml of serum. Where the SEM is not indicated it is within the compass of the symbol.

Text-fig.2. Effect of infection of chickens with NDV-Israel 1967 on serum ALP, AcP and GOT activities. Closed circles – infected chickens. Open circles – uninfected chickens. Mean enzyme activities (± SEM) expressed as milliunits per ml of serum. Where the SEM is not indicated it is within the compass of the symbol.
A dramatic change was observed in the level of alkaline phosphatase in the sera of infected chickens (Text-fig.2). When the birds were inoculated with NDV Israel 1967, there was a decrease of about 40% ($0.02 > p > 0.01$) in the level of ALP 48 hours after infection, which dropped sharply to a value of 132 milliunits per ml in comparison to 596 milliunits per ml in uninfected chickens (78% decrease, $p < 0.001$) at 72 hours post-inoculation. AcP levels in this serum were not changed by inoculation with the velogenic strain of virus. Significant changes were observed in transaminase activities when the chickens were inoculated with NDV Israel 1967 (Text-fig.2). GOT activity started to increase slightly 24 hours post-infection, and reached a value of 120 milliunits 72 hours after infection, an elevation of about 70% ($p < 0.001$). The level of glutamate-pyruvate-transaminase in chicken sera was found to be very low. This

**Text-fig. 3.** Effect of chickens with a mesogenic strain of NDV on serum LDH, ChE and ICDH activities. Closed circles – infected chickens. Open circles – uninfected chickens. Mean enzyme activities ($\pm$ SEM) expressed as milliunits per ml of serum. Where the SEM is not indicated it is within the compass of the symbol.

**Text-fig. 4.** Effect of infection of chickens with mesogenic strain of NDV on serum ALP, AcP and GOT activities. Closed circles – infected chickens. Open circles – uninfected chickens. Mean enzyme activities ($\pm$ SEM) expressed as milliunits per ml of serum. Where the SEM is not indicated it is within the compass of the symbol.
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has been previously recorded (Rivetz and Bogin, 1974). This may be explained by the low activity of this enzyme in various tissues of the fowl (Bogin and Israeli, unpublished observations).

Smaller changes in enzyme activities were observed in chicks infected with the mesogenic strain of NDV (Text-figs. 3 and 4). A slight, but significant increase (0.01 > p > 0.001) of the level of LDH was observed 168 hours post-inoculation (Text-fig. 3). A decrease of about 47% in ALP levels was found at 192 hours, rising at 240 hours, but still being considerably lower (22%) than that of control sera (0.05 > p > 0.02) (Text-fig. 4). No changes were observed in the activities of ChE, ICDH, AcP and transaminases (Text-figs. 3 and 4).

![Text-fig. 5. Effect of infection of chickens with NDV – La Sota on serum LDH, ChE and ICDH activities. Closed circles – infected chickens. Open circles – uninfected chickens. Mean enzyme activities (± SEM) expressed as milliunits per ml of serum. Where the SEM is not indicated it is within the compass of the symbol.](image1)

![Text-fig. 6. Effect of infection of chickens with NDV – La Sota on serum ALP, AcP and GOT activities. Closed circles – infected hickens. Open circles – uninfected chickens. Mean enzyme activities (± SEM) expressed as milliunits per ml of serum. Where the SEM is not indicated it is within the compass of the symbol.](image2)
As is shown in Text-figs. 5 and 6, infection of chicks with the La Sota strain of NDV caused no significant changes in enzyme activity.

The changes in enzyme levels observed in the course of infection with the virulent strain of NDV followed the viraemia that was found in the sera of inoculated chickens (Text-fig. 7). The viraemia reached its peak 48 hours post-inoculation and remained at this level or decreased slightly until death. The decrease of ChE and ALP levels occurred when the virus reached its peak in the blood, whereas the release of the enzymes LDH, ICDH and GOT started when the virus was first detected in the serum. No correlations were seen between the infectivity in blood and enzyme level changes with the other two viruses. The elevation of LDH and the decrease of ALP during infection with the mesogenic strain of NDV were observed after the serum virus titre had declined (168 hours). When the chicks were inoculated with the La Sota strain, no marked changes were found although a low infectivity was observed 120 hours post-inoculation.

**DISCUSSION**

Significant changes in enzyme levels were found following infection with the virulent strain of NDV – Israel 1967. These changes were more pronounced when high doses of virus were injected.

The enzyme most affected by viral infection was alkaline phosphatase, the serum level of which was relatively high (Rivetz and Bogin, 1974). Akün and Schulte (1953) reported an increase in the activity of this enzyme in chickens infected with NDV. Their findings do not agree with the results obtained in our experiments with the
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Furthermore, a similar decrease of about 80% in the activity of ALP was described by Lust and Squibb (1967). They showed a decrease 2-3 days post-inoculation, which was even more apparent 4-5 days after infection at the stage when the chicks showed clinical symptoms. They reported that the decrease in serum ALP was parallel to the drop of the enzyme level in the intestine, in contrast to a recorded increase of about 40% in the liver. These findings strengthen the assumption that the decrease in the levels of ALP is mainly due to damage in the intestine, since the predominant isoenzyme in plasma originates in the gut (Bide, 1970). Bide (1972) in a study of avian erythroblastosis, has shown that plasma ALP level was reduced as a result of both poor feeding and starvation. NDV infected chickens develop signs of reduced food consumption and intestinal activity followed by a reduction in body weight. The similarity in this phenomenon may indicate that the reduction in serum ALP level is partially due to a similar process.

The drop in the level of alkaline phosphatase was followed by a decrease in cholinesterase levels, but to a lesser extent. Infection of chicks with viruses of lower virulence did not bring about any change in the levels of this enzyme. Serum ChE and particularly pseudocholinesterase originate mainly in liver and are secreted into the blood. Very little is known about the levels of this enzyme in diseases of fowl. The decrease in ChE activity can be explained by a reduction in protein synthesis resulting from liver damage. Squibb showed that the changes in protein levels in liver can be affected both by the disease and by starvation resulting from anorexia (1964). The decrease in serum ChE might indicate that liver necrosis has occurred, which might also account for the elevated levels of enzymes in the serum.

Whereas the levels of ALP and ChE decreased at the terminal stage of the disease in the chickens infected with the velogenic strain, the level of LDH, GOT and to a lesser extent, ICDH increased 24 hours after the infection, presumably due to damage to the internal organs.

The results obtained in these experiments may be used to assess the degree of virulence of different strains of NDV. La Sota virus (the lentogenic strain) failed to cause changes in enzyme levels. Infection with the mesogenic strain resulted in moderate changes, particularly in the level of ALP, whereas the velogenic strain caused marked changes in several enzymes.

The changes in levels of enzymes were correlated with the gross pathological and histopathological changes in chickens infected with NDV. In earlier observations (Hornstein, unpublished observations) it was found that chicks exposed to the virulent virus (NDV Israel 1967) developed clinical signs such as depression, anorexia and dehydration, and finally became comatose. The damage found in organs and tissues might indicate that the strain is a viscerotropic type, i.e. the digestive system was mainly damaged, with ulceration of the intestine and haemorrhages, mainly in the proventriculus. It should be pointed out that in field cases, where the duration of the disease is longer, the virus shows a pantropic character and chickens develop neurologic signs as well. Similar findings were described with other virulent strains of NDV by Cheville and co-workers (1972). Such damage to organs and tissues can explain the enzymatic changes in the chicks inoculated with the velogenic strain. However gross and histological examination revealed no liver damage in spite of the changes in enzyme levels seen. Chickens that were infected with the commercial mesogenic virus, which is of a respiratory type, showed respiratory signs such as dyspnoea, and oedema of the eyelids. The slight damage in the respiratory tract and lungs which was found in the
chicks could partially account for slight increase in LDH levels in the serum. A more complete picture of the biochemical changes in tissues and organs of the infected fowl is needed. Experiments are in progress on this subject and in particular on the enzyme profile of a number of tissues taken from chicks infected with NDV.

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REFERENCES


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RESUME

Modifications biochimiques dans le sérum des poulets pendant l'infection avec des souches de virulences différentes du virus de Newcastle. I. Étude sur les enzymes.

Trois souches de différentes virulences du virus de la Maladie de Newcastle (NDV) ont été inoculées à des poulets agés de 5-6 semaines. Le taux sérique des enzymes: deshydrogénase-lactique (LDH), deshydrogénase-isocitrique (ICDH), cholinestérase (ChE), phosphatases alcaline et acide (ALP + AcP), transaminase glutamo-oxalacétique et glutamo-pyruvique (GOT + GPT), a été examiné.

On a trouvé une diminution significative du taux de la ChE et de l'ALP chez les poulets inoculés avec la souche vélogénique; Chez les mêmes poulets on a observé une augmentation du taux de l'LDH, de l'ICDH et de la GOT, mais le taux de l'AcP et de la GPT est resté invariable. Chez les poulets inoculés avec la souche mésogénique, on a constaté une faible diminution de l'ALP et en même temps une faible augmentation de l'LDH. On n'a trouvé aucun changement significatif chez les poulets inoculés avec la souche lentogénique.

Toutes les modifications décrites ci-dessus concordaient avec les signes cliniques des poulets inoculés.

ZUSAMMENFASSUNG

Biochemische Veränderungen in Hühnerseren, die im Verlauf einer Infektion mit Newcastle-Disease-Virus-NDV Stämmen unterschiedlicher Virulenz auftreten.

I. Untersuchung des Enzymgehaltes