Effect of Newcastle Disease on Serum Copper, Zinc, Cholesterol, and Carotenoid Values in the Chick

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Experimentally induced Newcastle disease virus infection of chicks, with a mortality index of 48%, was accompanied by increased concentrations in serum of copper and cholesterol and decreased concentrations of zinc and total carotenoids. These changes distorted, or were superimposed upon, the rhythmic variability in the normal serum concentrations of each of these moieties. Changing values for copper, zinc, and cholesterol became apparent before any overt signs of disease.

Infectious illnesses appear to influence the concentration of many normal constituents of serum. Of the trace elements studied, copper values generally increase, whereas those of zinc tend to decrease during bacterial or viral diseases (8; W.R. Beisel and R.S. Pekarek, Int. Rev. Neurobiol., in press). The reaction of serum cholesterol, on the other hand, is often variable in the presence of infection in that a depressed concentration has been observed during bacterial pneumonia, cholera, tuberculosis, viral infections, hepatitis, and malaria in man (1, 6). Increased cholesterol values have been reported during tuberculosis in man or Escherichia coli bacteremia in dogs, with normal values being maintained during gram-positive or gram-negative infections in man, or experimental yellow fever in monkeys (1, 7). Serum carotenoids tend to decrease during Newcastle disease virus (NDV) infection in the chick (12) and during active tuberculosis in man (4, 9).

Earlier studies have shown that the chick, infected with NDV, constitutes an excellent model for experimental attempts to relate biochemical responses resulting from infection with fluctuations resulting from daily biological rhythms (14, 15). The present report describes the effects of a standardized NDV infection in chicks on serum copper, zinc, cholesterol, and carotenoid concentrations.

MATERIALS AND METHODS

To insure adequate numbers for selection, approximately 500 White Leghorn male chicks were obtained at one day of age and housed in an isolated air-conditioned room with constant lighting; a commercial chick diet and water were offered ad lib. This regimen continued for 28 days and served to deplete the birds of any parental immunity to NDV. Body weights were taken weekly and only chicks meeting their genetic potential curve for full normal growth were used in the experiment.

Two days before the date set for NDV inoculation, at 8:00 AM, the chicks (28 days old) were divided into two study groups: a group to serve as noninfected controls and another to be infected experimentally with NDV. At the same time, five chicks were selected at random from each group and blood samples were obtained from them by cardiac puncture; the chicks were then discarded. The serum was separated and frozen for later analysis. This procedure was repeated at 3:00, 8:00, and 12:00 PM daily for 48 hr. After this period of preinoculation control collections, all chicks in the group designated for infection were inoculated (at 8:00 AM) with 0.1 ml of a 10^{-3} dilution of a Grun/Rutgers strain of NDV that showed an embryo lethal dose of 50% equal to 10^{-3} per 0.1 ml in 10-day-old embryos. Seventy inoculated chicks were reserved in separate cages to monitor disease progression and mortality. Blood samples continued to be taken from five control and five infected chicks at the above sampling times for the next six days; only living birds were sampled, and all were discarded after the samples were obtained. At the end of the NDV incubation period, 72 hr postinoculation, care was taken to insure that the five infected chicks selected for bleeding manifested a range of clinical findings characteristic of the NDV syndrome at the time of sampling (13).

In the analyses of the tissues, each sampling period and treatment were equally represented in any one run. Standard reference tissues and synthetic reference standards were also included. Total carotenoids were determined by the method of Bessey et al. (2); cholesterol was determined according to Bowman and Wolf (3), and the trace metals were determined by atomic absorption spectrophotometry (8).

Analyses of variance were performed as in Snedecor and Cochran (11). The average serum concentration for each moiety was determined from all samples from noninfected control chicks; this was ascribed a value of 100% and was used as the reference point for plot-
ting the percentage changes of control and infected groups at each sampling time.

**RESULTS**

At the end of the active involvement stage, mortality in the NDV reference group was 48%, confirming that the level of NDV involvement in this trial was severe (13).

Table 1 presents a statistical summary of the data analyzed over days postinoculation to show the effect of NDV on the serum components studied. Figure 1 depicts the sequential changes in serum cholesterol, carotenoids, zinc, and copper in control and NDV-infected chicks over the course of the entire observation period. Each point on the curve represents the average of 10 control chicks up to the time of inoculation and five control and five infected chicks thereafter. Time of inoculation is represented by the vertical dashed line.

**Cholesterol.** Cholesterol concentrations in the controls over the 8-day period averaged 139 ± 16 mg/100 ml (± standard deviation) but varied each day by as much as 27% of this value; the observed fluctuations did not conform to a precisely repetitive rhythm throughout each 24-hr period. Early in the incubation period in the NDV-infected chicks, cholesterol concentrations tended to become somewhat lower than those of the control group, but by 55 hr postinoculation and thereafter values were consistently higher (<0.05 to <0.01).

**Carotenoid.** Carotenoid values in the control chicks averaged 483 ± 86 µg/100 ml; they also showed nonrepetitive variability when compared to clock hours. There was a slight but sustained trend here toward the development of higher carotenoid concentrations throughout the 8 days of study. In the NDV-infected chicks, the carotenoid values began to decline soon after inoculation and concentrations continued to be lower than in control birds throughout the subsequent observation period (<0.05 to <0.01).

**Zinc.** Zinc values in the noninfected control chicks fluctuated up to 45% of the 8-day average of 166 ± 38 µg/100 ml within each 24-hr period, with troughs generally occurring at 3:00 or 8:00 PM and peaks at 8:00 AM or 12:00 AM. Very soon after inoculation with NDV, zinc values became depressed (<0.01) below those of the noninfected control birds. Zinc concentrations continued to be depressed (<0.01) throughout most of the incubation period, returned transiently to control levels during the initial stages of overt disease (active involvement), and then dropped significantly (<0.01) during the last 48 hr of the study.

**Copper.** Copper values averaged 16 ± 5 µg/100 ml in noninfected chicks and demonstrated clear-cut diurnal oscillations (<0.01). The oscillations, initially of small amplitudes, increased in magnitude after 48 hr of sampling and subsequently exhibited a 50 to 75% variability within each 24-hr period. This serum component, unlike the carotenoids, cholesterol, or zinc, thus showed a classical circadian rhythm throughout the entire 8-day observation period, with daily troughs occurring uniformly at 8:00 PM. An effect of the NDV infection on serum copper was not observed until 24 hr after inoculation when values started to increase. Subsequently values continued significantly (<0.01) above the controls throughout the remainder of the experiment. The daily variability of serum copper in the NDV-infected birds was great, but the pattern of fluctuations lost its clear circadian rhythmicity after inoculation.

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**Table 1. Summary of analysis of variance for serum cholesterol, carotenoids, zinc, and copper concentrations in control and NDV-infected chicks over 24-hr periods**

| Serum component | Time (hr) post-inoculation | F values |  |
|-----------------|---------------------------|---------|---|
|                 | NDV effect | Diurnal effect | NDV × diurnal |
| Cholesterol     | 0–24        | 2.32     | 0.49 | 0.73 |
|                 | 24–48       | 4.42a    | 0.64 | 2.39 |
|                 | 48–72       | 6.19a    | 5.94b | 2.07 |
|                 | 72–96       | 13.67b   | 2.57a | 2.35 |
|                 | 96–120      | 16.23b   | 2.39 | 1.94 |
|                 | 120–144     | 12.53b   | 2.58a | 1.99 |
| Carotenoids     | 0–24        | 3.98     | 3.59a | 0.55 |
|                 | 24–48       | 8.61b    | 1.53 | 0.47 |
|                 | 48–72       | 11.51b   | 1.68 | 0.79 |
|                 | 72–96       | 5.51a    | 1.30 | 0.19 |
|                 | 96–120      | 12.28b   | 2.12 | 1.37 |
|                 | 120–144     | 41.65b   | 0.71 | 2.14 |
| Zinc            | 0–24        | 11.77b   | 4.57b | 0.08 |
|                 | 24–48       | 38.17a   | 3.62a | 0.76 |
|                 | 48–72       | 13.63a   | 1.16 | 2.19 |
|                 | 72–96       | 0.73     | 3.15a | 1.94 |
|                 | 96–120      | 15.34a   | 4.01b | 2.22 |
|                 | 120–144     | 11.77b   | 2.43 | 0.12 |
| Copper          | 0–24        | 0.25     | 20.47b | 1.42 |
|                 | 24–48       | 30.79a   | 5.77b | 2.83a |
|                 | 48–72       | 31.75b   | 1.82 | 1.66 |
|                 | 72–96       | 30.30b   | 12.16b | 1.93 |
|                 | 96–120      | 18.78b   | 10.44b | 0.62 |
|                 | 120–144     | 64.55b   | 7.12b | 2.96a |

* P < 0.05.

b P < 0.01.
Fig. 1. Response of serum cholesterol, carotenoids, zinc, and copper during Newcastle disease of chicks. Each point represents the average of 10 birds before inoculation with virus and five control and five infected birds thereafter. Time of inoculation is represented by the vertical dashed line.
DISCUSSION

The present data indicate that an NDV infection (48% mortality index) of growing chicks is associated with increased values for serum copper and cholesterol and depressed values for serum zinc and carotenoids. These changes are similar to those reported to accompany a variety of bacterial or viral infections in mammalian species (1, 2, 4, 6–9).

The use of closely spaced serial collections in the present study allowed a correlation of biochemical changes with the timing of different stages of the NDV infectious process. For example, during the incubation period (0 to 72 hr), the depression in zinc and the increase in copper concentrations, and to a lesser extent total cholesterol, were true infection-related effects since dietary intake of the chicks remained normal at this time. The depression of total serum carotenoids, on the other hand, could have been influenced by the reduction of food intake which began 72 hr after inoculation with the virus.

Feigin et al. (5) observed in man that attenuated Venezuelan equine encephalomyelitis virus could alter the periodic circadian rhythm of whole blood amino acid concentrations, could markedly alter their absolute mean concentrations, or could do both, depending on the time of day that the attenuated virus was given as a vaccine. Rapaport et al. (10) found that daily periodicity in the urinary excretion of tryptophan metabolites was maintained in patients with Rocky Mountain spotted fever even though the absolute amounts excreted were greatly increased. The present study suggests that infection-related influences on both periodic rhythms and on deviations from a normal range of concentrations may vary from substance to substance during the same time period in a single infection.

The present data also emphasize the influence of periodicity on interpretation of results obtained from samplings of blood and other tissues. The diurnal oscillations observed here have a large magnitude of change, e.g., serum copper varies as much as 75% in 24 hr, to illustrate the size of potential error. If the concentration of a substance normally undergoes rhythmic changes during the course of an investigation, a set of values obtained at a single point in time cannot be employed as an acceptable control sample. Moreover, the diurnal patterns of the oscillations indicate that serum copper in normal chicks possesses a classical circadian rhythm; yet in the same milieu at the same time, zinc, carotenoids, and cholesterol apparently lack a definite pattern. This would suggest that diurnal changes of all serum constituents are not circadian or that some are more susceptible than others to Zeitgeber inputs. The significant changes in patterns caused by NDV at various periods of the disease cycle would lend support to the latter possibility.

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