Increase of Lactic Dehydrogenase and Neutralizing Antibody in Plasma of Chickens Infected with Rous Sarcoma Virus

(Accepted 15 March 1967)

Increased concentrations of plasma lactic dehydrogenase (LDH) were reported in chickens with clinical leukosis(1). These authors suggested that flocks could be screened, using this test, as had previously been done in bovine leukaemia(2). This paper reports the increase of plasma LDH activity in chickens experimentally infected with the Bryan high-titre strain of Rous sarcoma virus (BS-RSV). The results indicate its general unsuitability as a screening test in comparison with neutralizing antibodies.

White Leghorn cockerels from our own leukosis-free stock were inoculated intravenously with BS-RSV which had been extracted from a wing-web tumour. A 10 % (w/v) homogenate in 0.05 M-2-amino-2-hydroxymethylpropane-1,3-diol (tris) saline buffer pH 7.4 containing 10 % (v/v) calf serum and one unit hyaluronidase (Koch-Light Laboratories, Colnbrook, Bucks.) per ml. was stirred for 1 hr at 22°C. The extract was centrifuged at 200 g for 15 min, and the clear supernatant fluid was stored in a Linde liquid nitrogen refrigerator. The birds were bled from the heart and ethylenediamine tetracetic acid (EDTA) was added to give 10 mg./ml. From this blood smears were made, fixed in methanol and stained with May Grunwald-Giesma reagent. LDH assays were done (3) on the plasma obtained by centrifuging the blood at 3000 g. The reagents used were from the Sigma Chemical Co. Activity was expressed as units/ml. (4). Neutralizing antibody was assayed on the chorioallantois of 11-day-old chick embryos. A 1/5 dilution of plasma incubated at 56°C for 30 min. was mixed with an equal volume of tris buffer containing 500 pock forming units (p.k.f.u.) BS-RSV per ml. After incubating at 30°C for 1 hr 0.2 ml. of the mixture was inoculated on each of 6 eggs. A known negative plasma served as control. Pocks were counted after 7 days and plasma was assumed to contain antibody when the pock count was reduced to 70 % or less of the control.

Two experiments were done. In the first, four 6-week-old cockerels were inoculated with 10^6-8 BS/RSV and four remained as controls. In the second experiment, three 20-week-old cockerels were inoculated with 10^5-8 P.K.f.u. and three served as controls. Birds were bled thereafter at weekly intervals (or, if they were moribund, more frequently) and LDH and neutralizing antibody were assayed. All control birds remained healthy. LDH concentrations averaged, in Expts 1 and 2, 470 (220 to 720) and 310 (230 to 500) units/ml. Neutralizing antibody was not detected in any plasma. In six of the seven inoculated birds there was evidence of infection (Table 1). Five of these died; three had tumours in the viscera and evidence of circulating erythroblasts and myeloblasts and four of them had neutralizing antibodies. The exceptional bird (Expt 1, no. 1) evidently died before much antibody had formed. Bird no. 2 (Expt 1) survived and maintained a normal blood picture possibly because it had produced antibodies early; they were demonstrated on day 7. The LDH activity increased in four birds but the levels did not become abnormal (> 1000 units/ml.) until late, in general well after antibodies had been detected. It had been expected that by using
older birds and smaller inocula in Expt 2 the intervals between antibody formation, LDH increase and death would be prolonged; this expectation was fulfilled.

Abnormally high levels of LDH in the plasma have been described in patients with active neoplastic disease(5) and tumour-bearing mice(6). However, it was later shown that this abnormal enzyme activity was associated with a filterable agent which did not give rise to tumours(7). It seems from the results reported here that avian leukosis viruses do not themselves cause an increase in LDH, nor do they carry an associated virus which does so. It is possible that the activity is a product of neoplastic cells.

Table 1. Time of appearance of lactic dehydrogenase and neutralizing antibody in the plasma of cockerels infected with BS–RSV

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Dose per bird (pk.f.u.)</th>
<th>Bird no.</th>
<th>Days to death</th>
<th>First increase in LDH</th>
<th>First detection of neutralizing antibody</th>
<th>Tu- mours post mortem</th>
<th>Circulating erythroblasts and myelo- blasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (6-week-old birds)</td>
<td>$10^8$</td>
<td>1</td>
<td>13</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>$10^7$</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>$10^7$</td>
<td>20</td>
<td>20*</td>
<td>14</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>$10^7$</td>
<td>15</td>
<td>14</td>
<td>14</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>

| 2 (20-week-old birds) | $10^8$ | 1 | - | - | - | - | - |
| 3 | $10^7$ | 35 | 28 | 14 | + | + |
| 4 | $10^7$ | 35 | 28 | 14 | + | + |

*Time in days. All control birds (4 in Expt 1 and 3 in Expt 2) remained healthy and had no evidence of circulating blast cells or of tumours. In none did neutralizing antibodies appear and plasma LDH activity remained normal. Expt 1 lasted 28 days and Expt 2 35 days.

Avian leukosis viruses may be divided into two groups: those predominantly causing leukemia and those predominantly causing sarcomas. The former group may give a rapid rise in plasma LDH but the latter, of which BS–RSV is a member, evidently do not. We require for screening a test which detects all the leukoses; plainly, LDH levels do not provide a good basis for such a test since LDH activity is increased late in BS–RSV infection when the bird is virtually moribund and it would be much simpler to screen flocks for neutralizing antibody against a virus from each of the two major subgroups though this would have to be supplemented by COFAL(8) (com- plement fixation-avian leukosis) tests in groups of embryos to detect recent infections where antibody titres were still low.

The author wishes to thank Dr K. H. Fantes for his interest throughout the work and Mrs J. R. Smith for her technical assistance.

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REFERENCES


(Received 10 November 1966)