Antibody Production in Bursectomized Chickens Treated with Lipid and Protein Fractions from Bursa, Thymus and Liver

The suppression of antibody formation in chickens caused by surgical removal of the bursa of Fabricius at birth can be repaired by allogeneic bursal tissue implanted between visceral and parietal peritoneum[1] or by bursal grafts enclosed in diffusion chambers[2,3]. So far, there has been only 1 experiment in which extracts from bursa were used: a slight or insignificant stimulation of antibody production was obtained in bursaless chickens injected with saline extract of acetone-dried bursae[4]. The present study concerns the production of immune hemagglutinins in unoperated and neonatally bursectomized chickens following treatment with lipid and protein materials from the bursa, thymus and liver.

Bursae, thymic lobes and livers from 8-week-old Rhode Island Red chickens were homogenized separately in distilled water and lyophilized. One portion of dried tissue was treated with a mixture of chloroform and methanol (2:1) in Soxhlet apparatus. Organic solvents were then removed by vacuum distillation, and the lipid fractions thus obtained from the bursa, thymus and liver were homogenized in saline prior to the injection. Another portion of dried tissue was extracted in saline at room temperature, and the supernatant obtained after centrifugation was lyophilized. Dried protein fractions were dissolved in distilled water and used for injections. One group of Rhode Island Red chickens was bursectomized at birth, and another group of unoperated birds served as controls. At the age of 10 days, bursectomized and unoperated chickens were given the first 8 mg/ml i.p. injection of lipid or protein fraction, followed by further injections twice a week. Each chicken received a total of 10 mg of lipid or protein fraction before the immunization. Chickens of all groups (Table) were subsequently given 3 1 ml injections of a 10% suspension of human O red blood cells in the wing vein at intervals of 20 days. The sera were investigated within 5 days for the presence of hemagglutinins using 0.025 ml of serial dilution of serum and 0.025 ml of a 2% suspension of human O erythrocytes. The end-point of agglutinating activity was determined microscopically. Titers of mercaptoethanol-resistant antibody were also detected.

All bursectomized chickens were capable of making hemagglutinins after boosting[5]. Antibody production of bursectomized chickens treated with bursa lipid or thymus protein corresponded quantitatively to the immunological potential of bursaless birds injected with saline (Table). On the other hand, bursectomized chickens treated with bursa protein produced higher amounts of both ME-sensitive and ME-resistant hemagglutinins and this finding may indicate the humoral function of the bursa[1-4]. However, the antibody-producing capacity of birds thus treated did not surpass that of bursectomized chickens injected with thymus lipid, liver lipid or liver protein. The injection of lipid and protein materials from lymphoid tissues and liver had no effect on antibody formation in unoperated controls.

Although the experiments done with bursal tissue in millipore chambers[2,3] favour the idea that the bursa is capable of exerting a humoral function, the possibility still remains that some non-bursal factors might be contributing to the restoration of antibody production in bursectomized birds. For example, colon bacilli (dead or alive) and their products which are present in the lumen of the bursa probably act as a non-specific stimulus[6], especially when they are situated outside of their natural

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Effect of lipid and protein fraction isolated from bursa, thymus and liver on antibody production in bursectomized and unoperated chickens

Chickens treated with	No. of chickens	Mean peak hemagglutinin titer (log_2)					
		Primary response		Secondary response		Tertiary response	
		Pre-ME[a]	Post-ME[b]	Pre-ME	Post-ME	Pre-ME	Post-ME
Bursectomized chickens							
Saline	13	0	0	4.5 ± 2.47	1.6 ± 2.42	6.2 ± 3.62	2.6 ± 2.0
Bursa lipid	13	0.6 ± 1.38	0	4.7 ± 2.91	2.7 ± 3.14	6.0 ± 3.31	3.0 ± 2.48
Thymus lipid	14	2.0 ± 1.87	0	7.2 ± 1.09	4.4 ± 3.91	8.8 ± 2.68	5.9 ± 2.49
Liver lipid	10	1.2 ± 1.60	0	6.2 ± 0.74	4.0 ± 2.44	9.0 ± 0.66	4.1 ± 1.17
Bursa protein	13	1.1 ± 2.21	0	6.6 ± 2.13	3.5 ± 2.06	8.4 ± 1.28	5.7 ± 2.75
Thymus protein	12	0	0	5.8 ± 1.66	3.5 ± 2.87	6.7 ± 2.33	4.1 ± 2.89
Liver protein	8	1.8 ± 1.76	0	7.0 ± 1.06	6.1 ± 1.60	8.6 ± 1.12	7.9 ± 1.04
Unoperated chickens							
Saline	18	8.2 ± 0.90	6.3 ± 2.20	8.4 ± 1.34	7.3 ± 1.24	9.5 ± 1.26	8.5 ± 2.18
Bursa lipid	10	8.3 ± 0.60	7.0 ± 1.00	8.5 ± 0.80	7.8 ± 0.60	10.1 ± 0.83	9.8 ± 1.02
Thymus lipid	11	8.0 ± 0.92	6.0 ± 0.75	8.7 ± 0.91	8.3 ± 1.02	10.0 ± 0.75	9.7 ± 0.38
Liver lipid	10	7.5 ± 0.95	5.4 ± 1.18	8.8 ± 0.92	7.8 ± 0.92	10.0 ± 0.60	9.5 ± 0.70
Bursa protein	13	8.1 ± 0.66	6.5 ± 0.63	8.1 ± 1.31	7.6 ± 1.31	9.6 ± 0.83	9.2 ± 0.84
Thymus protein	9	8.1 ± 0.56	5.5 ± 0.81	8.3 ± 0.74	8.0 ± 0.66	9.3 ± 0.64	8.8 ± 0.91
Liver protein	10	7.9 ± 0.70	7.1 ± 0.94	8.8 ± 1.07	8.1 ± 0.76	10.3 ± 0.78	9.8 ± 0.60

[a] Pre-ME, pre-mercaptoethanol; [b] post-ME, post-mercaptoethanol.

environment. On the basis of the present results it might be supposed that the injected lipid and protein fractions from bursa, thymus and liver act as an adjuvant. However, the failure of bursa lipid and thymus protein to reconstitute and increase the antibody production in bursaless birds, and the inability of all the materials used to induce a higher elaboration of hemagglutinins in unoperated chickens does not support this assumption. The simplest construction which can be placed upon these results suggests that the multiple injections of some tissue constituents to a certain degree stimulate, in an unknown way, the 'quiescent' antibody-producing machinery of bursectomized birds, while being inactive in unoperated chickens whose antibody-forming apparatus is functioning normally. It is pertinent to mention here that we have put forward a hypothesis, in connection with the antibody response of hyperimmunized bursaless chickens[6], that the antibody response threshold is elevated in those birds and that cumulative exposure to antigens may trigger a response[7,8].

The most striking finding revealed by this study deals with the restorative activity of lipid and protein fractions from liver, the liver protein being particularly effective in reconstituting the formation of ME-resistant antibody. One might be tempted to infer that the liver per se is capable of exerting an immunological function or interfering in immune affairs. Bearing in mind the high functional and biochemical complexity of the liver, this possibility cannot be ruled out, although it requires more experimental support than that offered here[9].

Zusammenfassung. Neugeborene bursaektomierte Küken wurden mit aus der Thymus, Bursa fabricii und Leber isolierten Lipid- und Protein-Fraktionen behandelt und hernach durch menschliche Erythrocyten immunisiert. Einige dieser Fraktionen, einschliesslich der Leberproteine und Leberlipide, führen zu einer Erhöhung der Hämagglutinin-Produktion.

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Mechanism of Decreased Erythropoiesis in Thyroidectomized Rats

The thyroid clearly exerts an effect on red cell production, as is evidenced by the anaemia which develops in experimental animals and human subjects after thyroidectomy[1-5]. When the total circulating red cell volume reaches its new steady-state after thyroid ablation, the red cell survival has been found to be normal[4,5]. Therefore, anaemia develops as the result of a decreased red cell production.

Theoretically, a decrease in red cell production could result from several causes: such as a decrease in the number of erythrogenic elements in the blood-forming tissues, an increase in intramedullary time, or an increase in intramedullary death. Clarification of the mechanism by which red cell production is reduced in the thyroidectomized rat was the object of this study. For this purpose an iron kinetic study was done as well as determinations of number of erythroid cells in normal rats and rats of the same age injected with I^{131} 8 months prior to the onset of the study.

Male rats of the Wistar strain, weighing approximately 300 g were used throughout these studies. One half of the group was injected with 700 μC of I^{131} i.p. and the remainder served as normal controls.

The total circulating red cell volume was determined by the Fe^{59} labelled cell dilution method[6]. The results obtained at the time of autopsy are shown in the Table. Following thyroidectomy the circulating red cell volume decreased 24% below normal. Plasma volume showed an increase of 7%. Quantitative measurements of the fraction of the total erythropoietic marrow present in a femur obtained by in vivo labelling with Fe^{59} and isolation of the entire skeleton[7] showed that one femur contained 7.5% of the total erythroid marrow in normal rats, whereas this value was 6.3% in thyroidectomized rats.

For iron kinetic studies the rats were given 1.0 μC of Fe^{59} i.v. and at various times thereafter were anaesthetized with ether and as much blood as possible was drawn from the abdominal aorta. Through the same needle the rat was thoroughly perfused with saline (the jugular vein was cut to allow outflow of blood and perfusate) until the heart stopped beating. The detailed method has been published elsewhere[7].

After thyroid ablation, as it is shown in the Table, the plasma iron concentration decreased 15%. The rate of erythropoiesis as measured by the red cell iron turnover rate[8] decreased after thyroid destruction. The magnitude of the decrease in the hemoglobin synthesis rate was about 20% of the control value. A comparable decrease in the plasma iron turnover rate was found. The clearance half time of radioiron from the plasma was prolonged in the thyroidectomized group when compared to the normal one. The fractional decrease in the hemoglobin synthesis rate after thyroidectomy was similar to the decrease

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