Table 3. Variations in the reactivity of glycine max seed agglutinins absorbed with human red cells

Red cells	Number of samples tested	H_4GM +++	++	+	w+	H_5GM +++	++	+	w+	H_6GM +++	++	+	w+
Group A	13	9	2	2	–	13	–	–	–	12	1	–	–
Group B	22	11	3	7	1	19	2	1	–	10	5	4	3
Group AB	5	3	2	–	–	5	–	–	–	4	1	–	–
Group 0	25	–	–	–	–	–	–	–	–	–	–	–	–

separated from the lectin. 1 fraction of anti-A which was reactive with only human A and AB red cells, and other fraction of anti-B which was reactive for B and AB cells. No anti-H activity could be demonstrated in the lectin after it was absorbed with human red cells. Agglutination reactions of the absorbed lectin with human red cells (pooled) are shown in table 4. Belated appearance of agglutination with the anti-B fraction of the lectin supports our conclusion, arrived at earlier, that the absorbed lectin shows A-stressed activity.

Discussion. It is evident from the result of the present study that the agglutinatory principles in GM seed extract which bind the horse red cells are not identical with the principles that bind the receptors on the surface membrane of the human red cells, because the lectin, after it is completely absorbed with horse red cells, is still capable of agglutinating the human red cells, though selectively. The fact that the absorbed lectin gave specifically negative reaction with group-0 human red cells indicates that the absorbed fraction was anti-H. Since in all 6 samples tested, the lectin was deprived of its anti-H activity, leaving behind anti-(A+B), it appears that A-like and B-like antigen are lacking in horse. However, a more extensive screening of horse red cells is needed to establish this point beyond any doubt.

The 3 agglutinatory principles identified in the seeds of GM can be arranged in the order of their reactivity as under: anti-H > anti-A > anti-B. While anti-A and anti-B fractions can be separated by selective absorption with group-B and group-A human red cells respectively, it should be possible to secure the anti-H fraction through absorption elution technique from horse red cells or group-0 human red cells treated with the GM lectin.

Table 4. Reactions of glycine max seed agglutinins (absorbed with human red cells) against pooled red cells

Lectin	Absorbed with human red cells of group	Reactions of absorbed lectin against pooled red cells A	B	AB	0
Glycine max	A	–	– (1+)	– (2+)	–
	B	2+	–	2+	–
	AB	–	–	–	–
	0	3+	1+	2+	–

Reactions shown in parenthesis appeared after 40–45 min. Other reactions were observed within 15–20 min.

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Changes in the sialic acid content of chick thymus and bursa of Fabricius during age-involution[1]

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Summary. The sialic acid content of both thymus and bursa of Fabricius during their growth and involution phases in chick has been reported in this study. It is observed that the sialic acid concentration is very high in 1-week-old chickens. The concentration subsequently decreases to a significant level and rises again prior to the onset of involution. In the post-involution period, a more or less minimal and constant level is maintained. The role of sialic acid in cellular activities of thymo-bursal system has been discussed.

The thymus and bursa of Fabricius are 2 important primary lymphoid organs of birds. These organs have been involved with cell- and/or humor-mediated immunity[2,3]. Purified glycoprotein extract of calf thymus, rich in sialic acid, has been found to contain factors enhancing lymphopoiesis[4]. Reticular epithelial cells of the thymus are supposed to synthesize such materials[5]. The migration of glycoproteins of blood and 'homing' of lymphocytes to the peripheral lymphoid organs have been found to be associated with the presence or absence of sialic acid residues. Thus, it appears that sialic acid plays an important role in the cell- and humor-mediated immunity. In birds, since both bursa and thymus undergo age-involution[6], a quantitative study on the level of sialic acid during growth and involution may throw some light on the importance of this chemical component in thymic and bursal function.

Materials and methods. Rhodes Island Red chicks of different age groups (1 week, 4 weeks, 16 weeks and 24 weeks) from Government Poultry Farm, Ranaghat, W.B., were used in this experiment. The thymus and bursa from each individual were carefully removed and weighed immediately after dissection. Sialic acid was extracted and estimated employing 'thiobarbituric acid assay method' of Warren[7]. Absorbancies were determined at 550 nm. Using

Sialic acid concentration* of the thymus and the bursa of Fabricius of chicks of different age

Age (weeks)	Amount of sialic acid in µg/100 mg of tissue Thymus	Bursa of Fabricius
1	290.013 ± 9.164	183.565 ± 9.036
4	84.168 ± 3.154	196.148 ± 27.483
16	127.764 ± 4.389	118.577 ± 7.529
24	86.765 ± 2.527	114.241 ± 3.651

* Mean values from 6 determinations ± SE.

crystalline N-acetyl neuraminic acid (Sigma) as standard, a linear curve was obtained. The amount of sialic acid in the tissue sample was determined using the standard curve.

Results and discussion. Results documented in this study clearly indicate a change in the sialic acid content of both thymus and bursa of Fabricius of chicken during their normal age-involution. Thymic sialic acid is very high in 1-week-old and 16-week-old chickens (table). In the 4th and 24th weeks, there is a decline in the content of this component. Sialic acid concentration in the 1-week-old chicken bursa is also very high. It declines gradually during age, although the decrease is not so marked as that observed in the case of thymus. The thymus and bursa comprise the central lymphoid system in birds[6]. These organs are the sites of production of successive waves of lymphocytes which are transported to the peripheral lymphoid organs. It has been reported in chick thymus that these lymphocytes originate from a pool of immigrant stem cells, which are completely renewed once in the early postnatal period[8]. It is possible that, at this stage, there is a great demand for the polyanionic sialoglycoprotein, which is required to form a coating on the cell membrane for their attraction and final seeding into the lymphoid organs[9]. This mechanism justifies the presence of a very high content of sialic acid in the thymus and bursa of Fabricius of 1-week-old chicken. The growth of thymus in chicken reaches the peak on the 16th week of posthatching, after which involution is initiated[6]. A second rise of sialic in the thymus before involution, is perhaps directly associated with the outburst of mitotic activity and rapid transport of lymphocytes into circulation and other lymphoid organs. Before streaming out, these cells are coated at their surface with sialic acid, which aids in seeding to the proper peripheral lymphoid organs[9]. By contrast, sialic acid of the 4-week-old bursa did not show any decrease in its level. This is possibly due to the fact that the rate of growth of the bursa is faster than that of the thymus[6]. It is reported that in chicken the spontaneous age-involution of the bursa occurs long before the period of thymic involution[6]. In adult age, however, both these organs undergo involution. Our observations further reveal that sialic acid content is reduced to a minimal and constant level in the involuted thymus and bursa. It is known that the aging thymus of mammals continues its function at a basal level exhibiting renewal and seeding of lymphocytes at a very low rate[10]. These phenomena demand a supply of at least a small amount of sialic acid. Moreover, as thymic humoral factor in mammals has been found to be glycoprotein in nature, and as the reticuloepithelial cells of the thymic medulla (which proliferate greatly in the involuted thymus) are thought to be the origin of thymic humoral agent(s), the involvement of sialic acid in the biogenesis of such principles in young and aging thymus (and perhaps in bursa) cannot be ruled out.

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Radioimmunoassay of an invertebrate peptide hormone – the crustacean neurosecretory hyperglycemic hormone[1,2]

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Summary. Antibodies against hyperglycemic hormone (CHH) of *Carcinus* were raised in rabbits by injection of extract from sinus glands which contain high concentrations of CHH. The antiserum neutralizes the biological activity of CHH and binds ^{125}J-CHH. A RIA for CHH was established and was used to measure the hormone content of sinus glands.

In vertebrate endocrinology, the introduction of the radioimmunoassay has made possible the accurate determination of virtually all known hormones in physiological concentrations and has thus enormously extended our knowledge of the details of regulatory functions of hormones.

In invertebrates, RIAs have so far only been introduced for ecdysteroids[4] and juvenile hormone[5]. The ecdysteroid RIA, now established as a routine procedure, has greatly stimulated studies on the control of molting and metamorphosis in insects and crustaceans, and results have been obtained which were practically unattainable as long as the bioassay and gas chromatography were the only available techniques.

A wealth of evidence is available indicating the occurrence of a large variety of peptide hormones in invertebrates[6] which are vital for the regulation of many physiological processes. However, progress in this field has been slow, since determinations of hormones in tissues or blood still have to rely on sometimes laborious bioassays with limited accuracy and insufficient sensitivity. To our knowledge, no RIA has so far been reported for an invertebrate peptide hormone. This is mainly due to the fact that only very few hormones are available in pure form. Also, preparations are usually in short supply which makes it difficult to raise antibodies.

At present, isolation and complete structural elucidation, confirmed by synthesis, have been accomplished for 3 substances which may be regarded as hormones in the classical sense, namely crustacean red pigment concentrating hormone[7], retinal pigment activating hormone[8] and the insect adipokinetic hormone[9]. Another hormone which has been isolated and characterized in terms of amino acid composition is the neurosecretory hyperglycemic hormone from the eyestalk of decapod crustaceans[10,11] (CHH = crustacean hyperglycemic hormone). The CHH of *Carcinus* has recently been obtained in pure form from sinus glands and has been shown to be a 6700 dalton peptide containing 4 tyrosine residues[11]. It could therefore be expected to be susceptible to iodination with ^{125}J. It should be noted that none of