Comparative Study of the Glycosphingolipids of Chicken Bursa of Fabricius and of Chicken, Rat and Human Thymus

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1. The glycosphingolipid compositions of the thymus and bursa of Fabricius of young male chickens were compared. The two tissues were found to contain complex mixtures of both neutral glycosphingolipids and gangliosides. Both tissues contained mono-, di-, tri-, tetra- and penta-glycosylceramides; the pentaglycosylceramide displayed a reaction of identity with authentic Forssman antigen when tested against a specific anti-(Forssman antigen) serum. The ganglioside G_{M3} containing *N*-acetylneuraminic acid was the principal ganglioside of both tissues. 2. The thymus contained appreciable amounts of the simple ganglioside *N*-acetylneuraminylgalactosylceramide, a compound not found in the bursa. The ganglioside G_{D3} (disialohaematoside) was detected in both tissues. 3. Rat and human thymus, like sheep thymus [Narasimhan, Hay, Greaves & Murray (1976) *Biochim. Biophys. Acta* **431**, 578–591], both contained a tetraglycosylceramide species as their most complex neutral glycosphingolipid and possessed little or no Forssman antigen. They also contained a complex mixture of gangliosides. 4. The possible significance of these results is briefly discussed.

There have been a number of studies on the glycolipid composition of the circulating lymphocytes of several species (see Gottfried, 1972). Because of the central role of the thymus in T-lymphocyte development, two studies have also compared the glycolipids of circulating lymphocytes with those of their corresponding thymic tissues (Levis & Kesse-Elias, 1974; Narasimhan *et al.*, 1976). In all these studies, glycosphingolipids, comprising both neutral and ganglioside classes, were the only type of glycolipid detected in the cells and tissues analysed. Although the precise tissue origin of B-lymphocytes in mammals is still under study, the involvement of the bursa of Fabricius in B-lymphocyte development in birds appears firmly established (see Lydyard *et al.*, 1976). Droege *et al.* (1975) reported the results of a study of the carbohydrate composition of the glycoproteins of the surfaces of different types of chicken lymphocytes (including cells obtained from both thymus and bursa); their results suggested that the carbohydrate compositions of surface glycoproteins might be used as markers for different chicken lymphocyte populations. Glycosphingolipids are enriched on the

surfaces of cells in general (Gray, 1974) and on the surfaces of lymphocytes in particular (Hildebrand *et al.*, 1975). We therefore decided, in view of our previous work in this area (Narasimhan *et al.*, 1976) and of the findings of Droege *et al.* (1975), to examine the glycosphingolipid composition of chicken thymus and bursa to determine whether these tissues might exhibit different complements of these compounds. The results of such a study are reported here; some data on the glycosphingolipid profiles of rat and human thymus (tissues whose glycolipid compositions do not appear to have been previously described) are also included for purposes of comparison. The study has revealed that both chicken thymus and bursa of Fabricius exhibit generally similar profiles of glycosphingolipids; in particular, Forssman antigen was found to be the most complex neutral glycosphingolipid of both of these chicken tissues. In contrast, little or none of this compound was present in rat or human thymus, and instead a tetraglycosylceramide species was the most complex member of this class of glycosphingolipid in these tissues.

Materials and Methods

Source of tissues

Male chickens (4 weeks old) and adult male Wistar rats (150–200g body weight) were killed by decapitation. The thymus glands and bursae of the chickens and the thymus glands of the rats were carefully removed. Two samples of human thymus (from

Abbreviations used: the chromatographic migrations of the neutral glycosphingolipids resolved in this study are described relative to those of standard neutral glycosphingolipids, abbreviated as follows: glucosylceramide, GL-1a; galactosylceramide, GL-1b; lactosylceramide, GL-2a; digalactosylceramide, GL-2b; globotriaosylceramide, GL-3; globotetraosylceramide, GL-4; *N*-acetyl-galactosaminylglobotetraosylceramide (Forssman antigen), GL-5.

young male infants) were obtained at autopsy. All the specimens were stored at -70°C before extraction.

Glycolipid extraction

The thymus glands and bursae were carefully freed of connective tissue before analysis. Extraction of lipids from the tissues was performed by the method of Suzuki (1965). The upper- and lower-phase fractions of the resulting Folch extracts were subjected to mild alkaline methanolysis (Narasimhan *et al.*, 1976) to decrease contamination by alkali-labile lipids. Preliminary experiments revealed that this treatment did not affect the chromatographic migrations of any of the glycolipids detected in the different tissues studied. Both the lower- and upper-phase lipids were then fractionated separately by using silicic acid column chromatography, as previously described (Yogeeswaran *et al.*, 1972).

Analyses of glycolipids

Quantitative analyses of total ganglioside mixtures were performed by the resorcinol method of Svennerholm (1957), as modified by Miettinen & Takki-Luukkainen (1959). Portions of neutral glycosphingolipid and ganglioside fractions were subjected to t.l.c. on glass plates coated with silica gel G (250 μm thickness) in appropriate solvent systems for neutral glycosphingolipids and gangliosides (Narasimhan *et al.*, 1976). Aniline/diphenylamine reagent (Smith, 1960) was used to detect the neutral glycosphingolipids and resorcinol reagent (Wherrett & Cumings, 1963) to detect gangliosides. The quantitative distribution of sialic acid in individual gangliosides separated by t.l.c. was determined by the method of MacMillan & Wherrett (1969). Mild acid hydrolysis and neuraminidase treatment of certain gangliosides were conducted as described by Yogeeswaran *et al.* (1972) to determine the species of sialic acid and neutral glycosphingolipid contained in these compounds. The products of acid and enzyme hydrolysis were subjected to t.l.c. in a solvent system of chloroform/methanol/conc. NH_3 (sp.gr. 0.880)/water (25:20:1:5, by vol.); standards of *N*-acetyl- and *N*-glycolyl-neuraminic acids and of various standard neutral glycosphingolipids were applied to each chromatogram. The plates were sprayed first with resorcinol to detect the released sialic acids and residual gangliosides (if present) and then resprayed with aniline/diphenylamine reagent to detect the neutral glycosphingolipid products.

The sugar, fatty acid and long-chain base compositions of a number of the principal neutral glycosphingolipids of the chicken tissues were analysed by g.l.c. All analyses by this method were performed with a Hewlett-Packard model 5830-A instrument equipped with dual glass columns (234 cm $\times$ 2 mm internal diam.) and a flame-ionization

detector. The liquid support was 3% (w/w) OV-1 on acid-washed Chromosorb P (80–100 mesh) and helium was the carrier gas. For sugar and fatty acid analyses, portions of individual glycosphingolipids (purified by preparative t.l.c.) were subjected to acid methanolysis in methanol/HCl (0.75M) at 80°C for 20 h. Sugars were analysed as the trimethylsilyl ethers of their *O*-methyl glycosides (Vance & Sweeley, 1967) after acetylation of amino sugars (Clamp *et al.*, 1967; Yogeeswaran *et al.*, 1973). Fatty acids were analysed as their methyl esters (Yogeeswaran *et al.*, 1972). For analyses of long-chain bases, these compounds were liberated from individual glycosphingolipids by the method of Singh (1973). The presence of long-chain bases was first checked by t.l.c. in a solvent system of chloroform/methanol/water/conc. NH_3 (sp.gr. 0.880) (65:25:3:1, by vol.); the long-chain bases were detected by spraying with ninhydrin [1% (w/v) solution in acetone] (Carter & Gaver, 1967).

The concentrations of glycolipids were related to the protein contents of the non-lipid residues obtained during the initial lipid extraction; protein was measured by the procedure of Lowry *et al.* (1951), with bovine serum albumin as standard.

Immunodiffusion

The identity of the putative Forssman antigen isolated from the chicken thymus and bursa was established by using an antiserum raised in a rabbit to purified Forssman antigen from sheep erythrocytes. The antiserum and a sample of the purified Forssman antigen were generously supplied by Dr. S.-I. Hakomori, University of Washington, Seattle, WA, U.S.A. Details of the preparation of the antiserum have been described (Hakomori *et al.*, 1977). Analyses by immunodiffusion were performed on 1% (w/v) Ionagar no. 2 (Oxoid Ltd., London E.C.4, U.K.) slabs in Petri dishes. The centre wells were filled with the antiserum to the Forssman antigen and the outer wells with various serial dilutions of the purified thymic and bursal pentaglycosylceramide species and also with appropriate samples of the authentic Forssman antigen. Results were observed from 2 to 36 h after commencement of diffusion.

Standard glycolipids

Standard mixtures of neutral glycosphingolipids and gangliosides were isolated from human kidney and brain by the same procedures as described above for the isolation of the glycosphingolipids of chicken thymic glands and bursae.

Results

Fig. 1(a) shows the patterns of the neutral glycosphingolipids of chicken thymus (channel 2) and of chicken bursa (channel 3) as resolved by t.l.c. The

Fig. 1. Analysis by t.l.c. of the neutral glycosphingolipids of (a) chicken thymus and bursa of Fabricius and (b) rat and human thymus

Chromatography was performed in a solvent system of chloroform/methanol/water (65:25:4, by vol.). The chromatograms were sprayed with aniline/diphenylamine reagent. The compounds indicated by the white dots in certain channels were not glycolipids. The compounds indicated by GL-1, GL-2, GL-3, GL-4 and GL-5 corresponded in migration to mono-, di-, tri-, tetra- and penta-glycosylceramides respectively; a and b of GL-1 corresponded in migration to glucosyl- and galactosyl-ceramide respectively, and a and b of GL-2 corresponded in migration to lactosyl- and digalactosyl-ceramide respectively. Each glycosphingolipid resolved showed double-band resolution, although this was not prominent for pentaglycosylceramide. Results identical with those presented here were obtained from analysis by t.l.c. of three other preparations of each of chicken and rat thymus and chicken bursa of Fabricius and of one other preparation of human thymus. Key to samples: (a) 1, standard Forssman antigen (from sheep erythrocytes); 2, chicken thymus; 3, chicken bursa; 4, sheep thymus; (b) 1, standard mixture of neutral glycosphingolipids from human kidney and brain; 2, rat thymus; 3, human thymus; 4, standard Forssman antigen.

neutral glycosphingolipids of sheep thymus (previously characterized by Narasimhan *et al.* 1976) are shown for comparison. Compounds corresponding in migration to a mixture of glucosyl- and galactosylceramides (GL-1a and -1b respectively), to a mixture of diglycosylceramides (GL-2a and -2b) and to tetra- (GL-4) and penta- (GL-5) glycosylceramides are evident in both the chicken thymic and bursal extracts. Only traces of a glycosphingolipid corresponding in migration to triglycosylceramide are evident in both channels 2 and 3.

The chromatographic profiles of the neutral glycosphingolipids of rat thymus (channel 2) and of human thymus (channel 3) are shown in Fig. 1(b). The extract of rat thymus exhibits compounds corresponding in migration to mono- (GL-1a), di- (GL-2) and tetra- (GL-4) glycosylceramides. The human thymus exhibits a similar profile of neutral glycosphingolipids, but in addition compounds corresponding in

migration to galactosylceramide (GL-1b) and triglycosylceramide (GL-3) are evident. Both the rat and human thymus exhibited only traces of a compound corresponding in migration to Forssman antigen (GL-5).

Data on the carbohydrate composition and on the amounts of the principal neutral glycosphingolipids of chicken thymus and bursa and of rat thymus are presented in Table 1. With regard to the chicken thymus and bursa, the sugar analyses indicated the presence of both galactosyl- and glucosylceramides in these tissues. Similarly, the analyses of the glycolipids extracted from the total GL-2 (i.e. including both GL-2a and -2b) areas of preparative chromatograms indicated the presence of both galactosylglucosylceramide and digalactosylceramide. These findings were consistent with the multiple number of bands observed in the GL-1 and GL-2 areas of the chromatograms of these tissues (cf. Fig. 1a). The

Table 1. Amounts, carbohydrate compositions and tentative partial structures of the principal neutral glycosphingolipids of chicken thymus and bursa of Fabricius and of rat thymus

Analyses by g.l.c. were performed as described in the Materials and Methods section. Protein was measured by the method of Lowry *et al.* (1951). The amounts of the individual glycosphingolipids were calculated from their glucose contents (GL-1a, GL-2a, GL-4 and GL-5) or from their galactose contents (GL-1b and GL-2b). The GL-1a and -1b areas of the chromatograms were analysed together, as were the GL-2a and -2b areas; the amounts of the two glycosphingolipids in each of these two areas of the chromatograms were then calculated on the basis of the observed molar ratios. The results are the averages of duplicate analyses performed on each of two separate preparations of each glycosphingolipid. Abbreviations: Tr., trace; Cer, ceramide.

Chromatographic migration of the glycosphingolipid analysed	Content (nmol/mg of protein)	Molar ratio relative to Glc			Tentative partial structure
		Glc	Gal	GalNAc	
(a) Chicken thymus					
GL-1a	0.10	1	1.91	—	Glc-Cer
GL-1b	0.19				Gal-Cer
GL-2a	0.02	1	3.69	—	Gal-Glc-Cer
GL-2b	0.02				Gal-Gal Cer
GL-3	0.009	1	1.98	—	Gal-Gal-Glc-Cer
GL-4	0.02	1	1.70	0.72	GalNAc-Gal-Gal-Glc-Cer
GL-5	0.04	1	1.95	1.99	GalNAc-GalNAc-Gal-Gal-Glc-Cer
(b) Chicken bursa					
GL-1a	0.16	1	4.22	—	Glc-Cer
GL-1b	0.68				Gal-Cer
GL-2a	0.12	1	2.94	—	Gal-Glc-Cer
GL-2b	0.12				Gal-Gal-Cer
GL-3	0.008	1	1.90	—	Gal-Gal-Glc-Cer
GL-4	0.02	1	1.98	0.86	GalNAc-Gal-Gal-Glc-Cer
GL-5	0.18	1	1.97	2.32	GalNAc-GalNAc-Gal-Gal-Glc-Cer
(c) Rat thymus					
GL-1a	2.75	1	Tr.	—	Glc-Cer
GL-2a	0.15	1	1.08	—	Gal-Glc-Cer
GL-4	0.25	1	1.91	0.78	GalNAc-Gal-Gal-Glc-Cer

Fig. 2. Reactivity of the pentaglycosylceramide species of chicken bursa of Fabricius and thymus with an anti-(Forssman antigen) serum as studied by immunodiffusion in an Ouchterlony plate

Wells contained: 1 and 4, standard Forssman antigen (from sheep erythrocytes); 2, chicken bursa pentaglycosylceramide; 3, chicken thymus pentaglycosylceramide; 5, bovine serum albumin; 6, 0.9% NaCl. The central well contained the anti-(Forssman antigen) serum. The plate was photographed 24 h after commencement of immunodiffusion.

analyses by g.l.c. of the sugar composition of the glycolipid of both chicken thymus and bursa that corresponded in migration to Forssman antigen revealed Glc:Gal:GalNAc in the proportions of approx. 1:2:2; these values are consistent with the interpretation that this component was indeed a species of Forssman antigen. The sugar analyses of the glycosphingolipids of rat thymus suggest that the principal neutral glycosphingolipids of this tissue are glucosylceramide, galactosylglucosylceramide and an aminoglycolipid (globoside) species containing *N*-acetylgalactosamine.

The availability of a specific antiserum to Forssman antigen permitted us to determine whether the pentaglycosylceramide species of both chicken thymus and bursa cross-reacted with this antiserum. The results of an analysis of this nature performed on the pentaglycosylceramide species of these tissues are shown in Fig. 2. The centre well contained the antiserum to the pure Forssman antigen. Samples of the purified bursal and thymic pentaglycosylceramides were applied in wells 2 and 3 and of the standard Forssman antigen in wells 1 and 4. Both the bursal and thymic pentaglycosylceramides showed lines of

Table 2. Fatty acid composition of the neutral glycosphingolipids of chicken thymus and bursa of Fabricius. The results are expressed as percentages of the total fatty acids recovered and are the averages of duplicate analyses on each of two different preparations of each lipid. Abbreviation: Tr., trace.

Glycosphingolipid ... Fatty acid	GL-1a and -1b		GL-2a and -2b		GL-3		GL-4		GL-5	
	Thymus	Bursa	Thymus	Bursa	Thymus	Bursa	Thymus	Bursa	Thymus	Bursa
C ₁₄ :0	Tr.	1.0	Tr.	1.0	Tr.	3.1	0.2	1.4	—	—
C ₁₆ :0	27.2	26.9	27.3	27.4	12.9	13.9	14.2	21.1	10.1	7.6
C ₁₆ :1	1.6	2.8	1.2	2.2	Tr.	1.2	1.0	2.4	0.3	0.1
C ₁₈ :0	19.1	11.4	18.9	16.5	20.1	19.5	14.2	15.6	8.0	9.9
C ₁₈ :1	11.4	28.1	11.9	20.9	17.7	22.2	13.3	20.3	2.2	0.9
C ₂₀ :0	12.8	6.9	9.9	5.3	26.0	23.1	9.7	4.2	11.9	16.1
C ₂₀ :1	1.9	3.9	3.8	3.5	Tr.	—	7.9	17.9	Tr.	Tr.
C ₂₂ :0	25.4	11.3	13.4	12.3	19.9	13.9	16.5	13.1	23.1	17.7
C ₂₂ :1	—	—	9.7	—	Tr.	—	5.9	—	—	—
C ₂₄ :0	0.6	5.6	3.9	7.5	3.4	3.1	8.8	4.0	22.9	20.6
C ₂₄ :1	Tr.	2.1	Tr.	3.4	Tr.	Tr.	8.3	Tr.	21.5	27.1

immunoprecipitation with the antiserum and displayed a reaction of complete identity with each other and with the standard Forssman antigen. No reaction of the antiserum was observed with bovine serum albumin (well 5) or 0.9% NaCl (well 6). Essentially similar results were obtained in three other analyses; on no occasion was spurring of the immunoprecipitation zones observed.

The results of analyses by g.l.c. of the fatty acid compositions of the principal neutral glycosphingolipids of chicken thymus and bursa are shown in Table 2. As shown, a variety of fatty acids, varying in chain length from 14 to 24 carbon atoms, were detected in the individual glycosphingolipids. Analyses by g.l.c.-mass spectrometry (kindly performed by Mr. L. Marai and Dr. A. Kuksis, Banting and Best Department of Medical Research, University of Toronto) revealed that no hydroxy fatty acids were present in the principal neutral glycosphingolipids of the chicken tissues.

Analyses of the long-chain base composition of the four principal neutral glycosphingolipids of chicken thymus and bursa were also performed by g.l.c. Sphingosine was the principal long-chain base in all of the compounds examined; lesser amounts of another long-chain base, tentatively characterized as the C₂₀ analogue of sphingosine, were also detected.

The results of quantitative determinations of the total ganglioside contents of chicken thymus and bursa and of rat and human thymus are shown in Table 3; chicken bursa contained appreciably more ganglioside (expressed as nmol/mg of protein) than chicken thymus, and the rat and human thymus contained appreciably less ganglioside than either of the two chicken tissues.

Analyses by t.l.c. of the gangliosides of chicken thymus (channel 2) and bursa (channel 3) are shown in Fig. 3(a). Both tissues contain a complex mixture of gangliosides, exhibiting compounds corresponding in

migration to the gangliosides G_{M3}, G_{M2}, G_{M1}, G_{D1a} and also to more complex gangliosides. In addition, the chicken thymus exhibited appreciable amounts of a very-fast-migrating resorcinol-positive compound, corresponding in chromatographic migration to the *N*-acetylneuraminylgalactosylceramide species of human brain (indicated by X on the left-hand margin of channel 1). The ganglioside pattern of rat thymus is shown in Fig. 3(b) (channel 1); the pattern is complex, exhibiting components corresponding in migration to most of the gangliosides detected in the standard human brain mixture (channel 2). A comparison of the ganglioside profiles of human (channel 1) and sheep (channel 2) thymus [the ganglioside profile of this latter tissue was reported previously by Narasimhan *et al.* (1976)] is shown in Fig. 3(c). The principal gangliosides in the human preparation correspond in migration to gangliosides G_{M3}, G_{M1} and G_{D1a}.

Quantification of the contribution of individual gangliosides to the total patterns resolved by t.l.c. was performed by the technique of MacMillan & Wherrett (1969). The results of these analyses are shown in Table 3. In general, those analyses confirmed that the ganglioside corresponding in chromatographic migration to the ganglioside G_{M3} was the principal ganglioside of all the tissues studied.

The results of carbohydrate analyses designed to characterize partially the principal gangliosides of the chicken tissues and of rat thymus are summarized in Table 3. From these analytical data, tentative partial structures for the principal gangliosides of these tissues are proposed in Table 3.

Discussion

These studies have revealed that chicken thymus and bursa of Fabricius both exhibit quite complex and rather similar profiles of glycosphingolipids. A major

Table 3. *Results of ganglioside analyses*

(a) Ganglioside content. Ganglioside sialic acid and protein were measured as described in the Materials and Methods section. The numbers of preparations analysed are shown in parentheses; each analysis was performed in duplicate. The results are means $\pm$ s.d. (b) Percentage distribution of sialic acid in the principal gangliosides. The distribution of sialic acid in the individual ganglioside zones separated by t.l.c. was determined by the method of MacMillan & Wherrett (1969). The results are expressed as percentages of the total sialic acid recovered (at least 80% of that applied in all cases) and are not corrected for the molar content of sialic acid of each ganglioside. The areas of the chromatograms containing the more complex gangliosides (G_{D1a} to G_T) were analysed together. The numbers of preparations analysed are given in parentheses. The results are means $\pm$ s.d., except for rat thymus, where they represent the mean only. The ganglioside nomenclature is that of Svennerholm (1964). (c) Results of partial-characterization studies of the principal gangliosides. The conditions of mild acid hydrolysis and neuraminidase treatment were as described by Yogeeswaran *et al.* (1972). The molar ratios of the carbohydrates were determined by g.l.c. and represent the means of duplicate determinations. Abbreviation: Cer, ceramide.

(a)		Lipid-bound sialic acid ($\mu\text{g}/\text{mg}$ of protein)					
Sample							
Chicken thymus (4)		0.34 $\pm$ 0.02					
Chicken bursa (4)		0.62 $\pm$ 0.01					
Rat thymus (4)		0.10 $\pm$ 0.02					
Human thymus (2)		0.18					
(b)							
Ganglioside	...	AcNeu-Gal-Cer	G_{M3}	G_{M2}	G_{M1}	G_{D3}	G_{D1a} to G_T
Sample							
Chicken thymus (4)		6.8 $\pm$ 1.2	19.6 $\pm$ 3.6	3.9 $\pm$ 1.9	12.7 $\pm$ 1.1	25.4 $\pm$ 2.1	31.6 $\pm$ 6.7
Chicken bursa (4)		—	27.4 $\pm$ 2.5	7.5 $\pm$ 1.2	7.3 $\pm$ 1.3	14.7 $\pm$ 2.5	43.1 $\pm$ 4.9
Rat thymus (2)		—	16.5	11.2	15.3	—	57.0
(c)							
Chromatographic migration of the ganglioside	Products of:		Molar ratios relative to glucose			Tentative partial structure	
	Mild acid hydrolysis	Neuraminidase treatment	Glc	Gal	Sialic acid		
(1) Chicken thymus Sialylgalactosyl- ceramide	GL-1b, AcNeu	GL-1b, AcNeu	—	1.0	0.87	AcNeu-Gal-Cer	
G_{M3}	GL-2a, AcNeu	GL-2a, AcNeu	1.0	1.02	1.17	AcNeu-Gal-Glc-Cer	
G_{M1}	GL-4, AcNeu	No effect	Not analysed			AcNeu-AcNeu-Gal-Glc-Cer	
G_{D3}	GL-2a, AcNeu	GL-2a, AcNeu	1.0	0.82	1.85		
(2) Chicken bursa							
G_{M3}	GL-2a, AcNeu	GL-2a, AcNeu	1.0	0.99	1.13	AcNeu-Gal-Glc-Cer	
G_{M1}	GL-4, AcNeu	No effect	Not analysed			AcNeu-AcNeu-Gal-Glc-Cer	
G_{D3}	GL-2a, AcNeu	GL-2a, AcNeu	1.0	0.98	1.50		
(3) Rat thymus							
G_{M3}	GL-2a, AcNeu	GL-2a, AcNeu	Not analysed				
G_{M1}	GL-4, AcNeu	Not analysed	Not analysed				

finding with respect to the neutral glycosphingolipids of these tissues was the detection in both of a pentaglycosylceramide species that showed a reaction of complete identity with an authentic sample of Forssman antigen when tested with an antiserum to this pure lipid (Fig. 2). This finding is perhaps not entirely unexpected, as the chicken is a Forssman-positive species, and immunohistochemical studies (Tanaka & Leduc, 1956) have demonstrated the presence of this compound in a number of other tissues of this bird. Another finding of interest with

respect to the neutral glycosphingolipids was the detection of galactosyl- and galactosylgalactosylceramides in both chicken tissues. The presence of these glycosphingolipids in extraneural tissues has been described previously (see Mårtensson, 1969), but they occur less commonly than glucosyl- and lactosylceramides. The fatty acid compositions of the neutral glycosphingolipids (Table 2) also merit comment; in general, the more complex neutral glycosphingolipids exhibited a higher content of long-chain fatty acids, until species of C_{22-24} chain

Fig. 3. Analysis by t.l.c. of the gangliosides of (a) chicken thymus and bursa of Fabricius, (b) rat thymus and (c) human and sheep thymus

The chromatograms were developed in a solvent system of chloroform/methanol/conc. NH_3 (sp.gr. 0.880)/water (60:35:1:7, by vol.) and sprayed with resorcinol reagent. The white dots in channels 1 of (b) and (c) indicate compounds that were not gangliosides. Results identical with those presented here were obtained from analysis by t.l.c. of three other preparations of each of chicken and rat thymus and chicken bursa of Fabricius and of one other preparation of human thymus. X, *N*-acetylneuraminylgalactosylceramide; G_{D_3} , disialohaematoside. The nomenclature of the gangliosides is that of Svennerholm (1964). Key to samples: (a) 1, standard mixture of gangliosides of human brain; 2, chicken thymus; 3, chicken bursa; (b) 1, rat thymus; 2, standard mixture of gangliosides of human brain; (c) 1, human thymus; 2, sheep thymus.

length comprised approx. 65% of the total in the Forssman species; the preponderance of long-chain fatty acids in Forssman antigen has been observed previously (Mallette & Rush, 1972; Naiki *et al.*, 1972).

Both chicken tissues displayed complex profiles of gangliosides. Several aspects of this observation merit comment. Firstly, the ganglioside G_{M_3} containing *N*-acetylneuraminic acid was the principal ganglioside of both tissues, in accordance with the general occurrence of this compound as the major ganglioside of extraneural tissues (Wiegandt, 1971). Secondly, the finding of a compound exhibiting the properties of disialohaematoside (ganglioside G_{D_3}) is somewhat unusual; this ganglioside has been detected in other tissues (e.g. retina and kidney) (Wiegandt, 1971), but its occurrence is quite restricted. Thirdly, the presence of *N*-acetylneuraminylgalactosylceramide in chicken thymus is also rather novel, as this simple ganglioside has previously only been reported in brain (Wiegandt, 1971). However, like disialohaematoside, it is certainly not a universal constituent of thymus, as we have not observed the presence of either of these compounds in rat or human thymus (the present study) or sheep thymus (Narasimhan *et al.*, 1976).

Both rat and human thymus also exhibited complex profiles of neutral glycosphingolipids and gangliosides. Perhaps the most significant finding

with respect to these tissues was that a tetraglycosylceramide species (shown in rat thymus to be an aminoglycolipid or globoside species) was the most complex neutral glycosphingolipid of these tissues, and that they contained little or no Forssman antigen. This latter finding is consistent with the fact that both of these species are generally Forssman-negative (Buchbinder, 1935). There appears, however, to be no simple correlation between Forssman status and the presence of this antigen in thymus as the most complex neutral glycosphingolipid; the sheep, whose erythrocytes contain Forssman antigen, has little or none of this compound in its thymus (cf. Fig. 3) and instead tetraglycosylceramide is the most complex neutral glycosphingolipid of this tissue (Narasimhan *et al.*, 1976).

The major finding of the present study has been the complexity of the profiles of glycosphingolipids exhibited by the tissues studied, particularly those of the chicken. It appears unlikely that this observation has any particular immunological significance. More likely, the presence of complex profiles of glycosphingolipids in the tissues studied here probably reflects the presence in these tissues of various receptor or functional activities with which glycosphingolipids are associated (Gray, 1974). Certainly, a number of other non-lymphoid tissues, such

as human testis (Levine *et al.*, 1976), also exhibit extremely complex profiles of glycosphingolipids.

A number of questions that could be the subject of future investigations are suggested by the results obtained here. For instance, what is the cellular location of the Forssman antigen in chicken thymus and bursa? If in lymphoid cells, do circulating B- and T-cells both contain this antigen or is it lost during the processes of differentiation involved in the formation of these mature cells? Also, if present in lymphocytes, will it be possible to study the capping and lateral mobility of this endogenous glycolipid in the membranes of these cells in a manner analogous to that used for an exogenous glycolipid (Revesz & Greaves, 1975)? Finally, do all the thymus glands of higher animals contain either penta- or tetra-glycosylceramides as their most complex neutral glycosphingolipids? The use of specific fluorescent antibodies to these glycosphingolipids (cf. Marcus, 1976) provides one approach to answering some of these questions.

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