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Serum Gamma Globulin Levels and the Detection of IgG Heavy Chain and Light Chain in the Serum and Urine of Cases of Pig Hereditary Lymphosarcoma*

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Abstract—*Pigs with hereditary lymphosarcoma were found to show an increase in serum gamma globulin levels from* 0.24 *to* 2.04 g/dl *during a period from the* 10*th to the* 24*th week of life. Compared to normal litter mates this increase was significant* ($P < 0.01$). *The increase was due to IgM and IgG and was accompanied by a fall in serum albumin.* ($P < 0.01$).

All cases in the later stages of the disease had an IgG heavy chain (γFc) *and light chain* (γL) *component in their serum and urine. This component could not be found in the serum or urine of normal litter mates, but could be extracted from mesenteric lymph nodes of normal pigs and lymphosarcoma cases. The molecular size of the main component in serum and urine was approximately* 50 *to* 60×10^3. *Other tumour cell products similar to foetal albumin, alpha*$_2$ *and beta globulin were also present in the serum of lymphosarcoma cases. With the exception of trace amounts of albumin foetal proteins were not present in the serum of normal litter mates.*

INTRODUCTION

REPORTS on the clinical signs, haematology, pathology and genetics of an hereditary lymphosarcoma of pigs have already been published [1, 2]. Campbell [3] reported on the ultrastructure, cell cultures and cell enzymology of the condition. The effect of prednisolone therapy on these animals has been reported by Brownlie *et al.* [4].

The disease is present in a breeding herd of Large White pigs and its appearance is controlled by an autosomal recessive gene. Affected animals develop multicentric tumours of the lymph nodes, particularly the gastrosplenic, mesenteric and bronichial lymph nodes and have an associated lymphocytic leukaemia with terminal anaemia and thrombocytopenia. The disease can be detected as early as 6–12 weeks of age and cases have an average life span of 4–6 months with a few surviving 15–18 months. This communication describes a study of the serum proteins, urine and mesenteric lymph nodes of lymphosarcoma cases and normal litter mates.

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MATERIALS AND METHODS

Serum obtained from lymphosarcoma cases was used fresh when possible but samples were stored at −60°C and used as and when necessary. Total proteins were estimated by the biuret method as adapted by Henry *et al.* [5].

Gel diffusion, electrophoresis and immunoelectrophoresis were carried out on microscope slides using 1% agar (Difco, "Special Agar Noble") in the discontinuous veronal/calcium lactate buffer system, pH8.6 of Hirshfield [6]. Further electrophoresis was performed on Millipore® Phoroslide membranes. After staining with Ponceau S, they were dehydrated and cleared, then quantified on a Phoro Scope densitometer.

Table 1. *Mean values of serum albumin, alpha-, beta- and gamma-globulin and total proteins in a total of* 13 *cases of lymphosarcoma* (LS) *with normal litter mates during a period from* 8 *to* 30 weeks *of age. Numbers of animals sampled are shown in brackets*

Age in weeks	Albumin		α-globulin		β-globulin		γ-globulin		Total protein		Number sampled	
	LS	Normal	LS	Normal	LS	Normal	LS	Normal	LS	Normal	LS	Normal
8	2.8	2.8±0.4	0.7	0.7±0.3	0.6	0.6±0.3	0.1	0.1±0.1	4.2	4.1±0.6	(1)	(9)
10	3.4	3.3±0.4	0.9	0.7±0.2	0.6	0.7±0.2	0.2	0.1±0.1	5.1	4.7±0.3	(1)	(9)
12	2.4±0.5	2.9±0.5	1.1±0.1	0.9±0.3	0.8±0.1	0.9±0.3	0.6±0.1	0.2±0.2	4.7±0.6	5.0±0.5	(3)	(13)
14	2.4±0.4	3.3±0.5	1.2±0.1	0.8±0.4	1.1±0.1	0.8±0.2	0.6±0.4	0.4±0.3	5.3±0.8	5.2±0.6	(3)	(11)
16	3.0±1.1	3.4±0.6	1.0±0.3	0.8±0.3	0.7±0.2	0.7±0.1	1.1±0.2	0.3±0.2	5.6±1.1	5.1±0.4	(3)	(13)
18	2.5±0.1	3.3±0.5	1.2±0.3	0.7±0.2	1.3±0.1	0.8±0.1	1.2±0.6	0.4±0.3	6.2±0.7	5.3±0.4	(3)	(11)
20	3.3±0.9	3.2±0.5	0.6±0.7	0.8±0.3	0.8±0.6	1.0±0.2	1.5±0.6	0.4±0.3	6.2±0.6	5.4±0.6	(3)	(11)
22	2.7	3.0±0.3	1.5	1.1±0.3	1.0	1.1±0.2	1.6	0.5±0.3	6.8	5.7±0.4	(1)	(6)
24	3.2	3.4±0.5	1.2	0.7±0.1	1.2	1.0±0.2	2.0	0.3±0.4	7.5	5.6±0.3	(1)	(4)
26–30	2.6±0.6	3.3±0.2	1.0±0.4	1.0±0.1	1.1±0.1	0.9±0.3	1.7±0.3	0.3±0.2	6.4±0.5	5.5±0.4	(6)	(3)

Table 2. *Results of a* t*-test comparison of mean values for serum protein levels in* 14 *lymphosarcoma pigs and a similar number of randomly selected normal litter mates sampled at the same age*

Serum protein	Lymphosarcoma pigs			Normal pigs			*t*-value
	Mean	S.D.	S.E.	Mean	S.D.	S.E.	
Albumin	2.41	0.61	0.37	2.96	0.28	0.08	3.07**
Alpha-globulin	1.08	0.21	0.04	1.10	0.21	0.04	0.25 NS
Beta-globulin	1.26	0.36	0.13	1.19	0.21	0.05	0.63 NS
Gamma-globulin	1.20	0.75	0.56	0.51	0.29	0.08	3.21 **
Total protein	5.96	0.92	0.85	5.75	0.37	0.14	0.79 NS

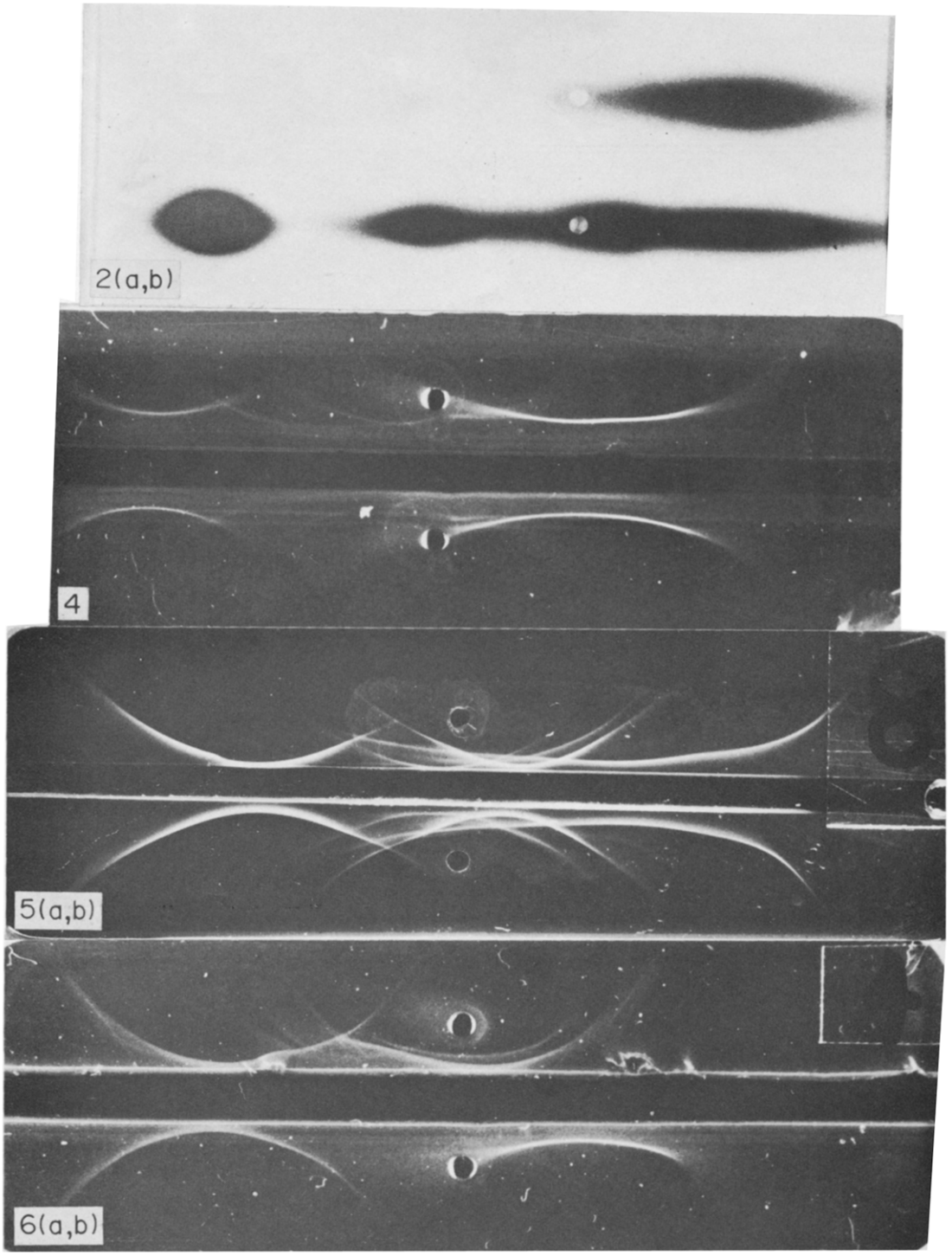

Fig. 2. Electrophoresis of (a) an extract from the 2nd peak of a Sephadex G200 fractionation of serum from a lymphosarcoma pig, and (b) whole serum from the same animal.

Fig. 4. IE of the 4th peak extract from a Sephadex G200 fractionation of lymphosarcoma serum run against rabbit antiserum to 4th peak fraction.

Fig. 5. IE of (a) lymphosarcoma whole serum and (b) normal whole serum against 4th peak antiserum.

Fig. 6. IE of (a) foetal pig sera and (b) Sephadex G200 4th peak fraction of lymphosarcoma serum against 4th peak antiserum.

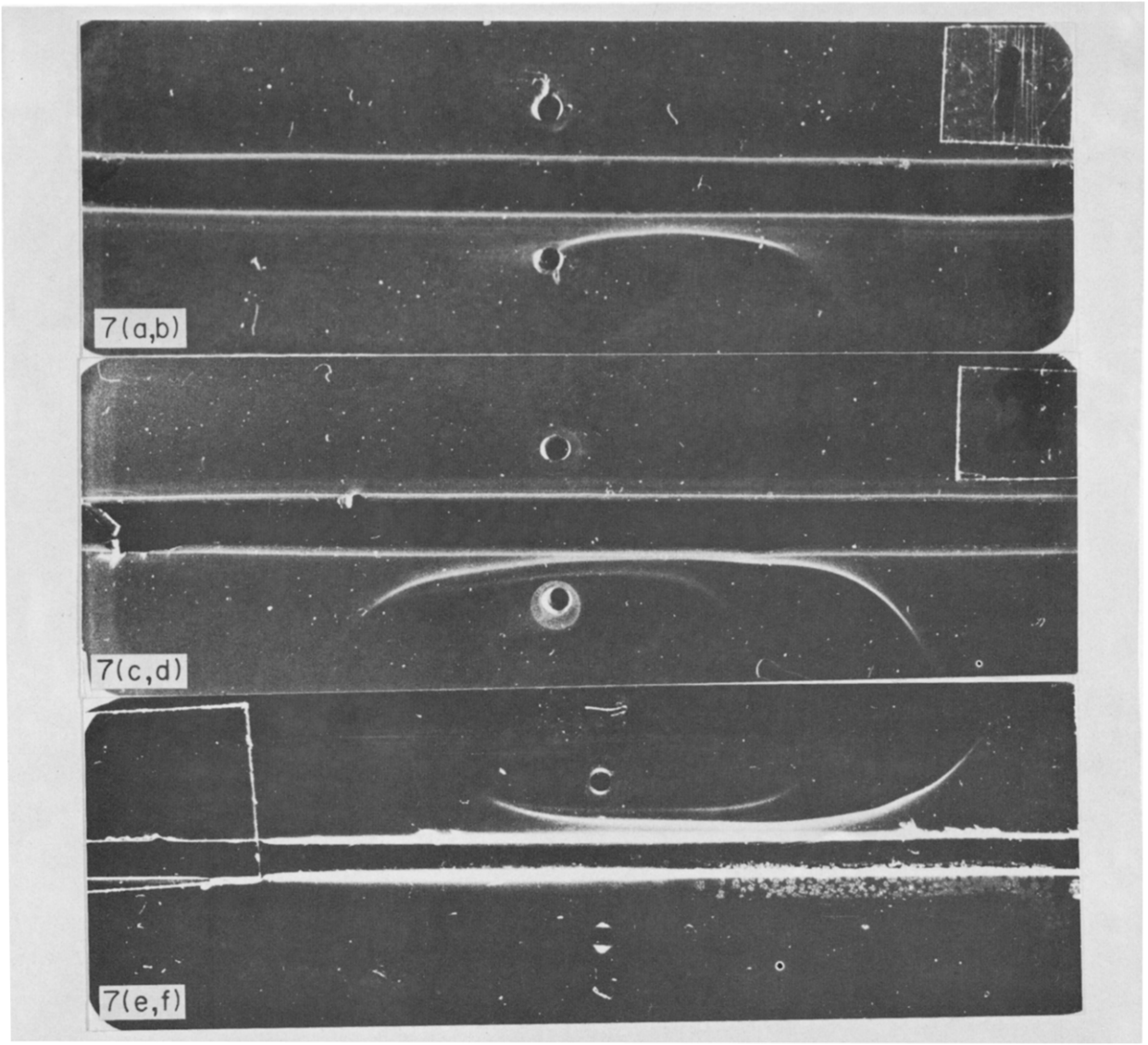

Fig. 7. IE of (a) and (c) FPS (b) 4th peak fraction from a lymphosarcoma pig (d) whole lymphosarcoma serum (e) whole normal serum (f) 4th peak fraction from a normal pig all against 4th peak antiserum absorbed with FPS in 1, 2 *and* 3.

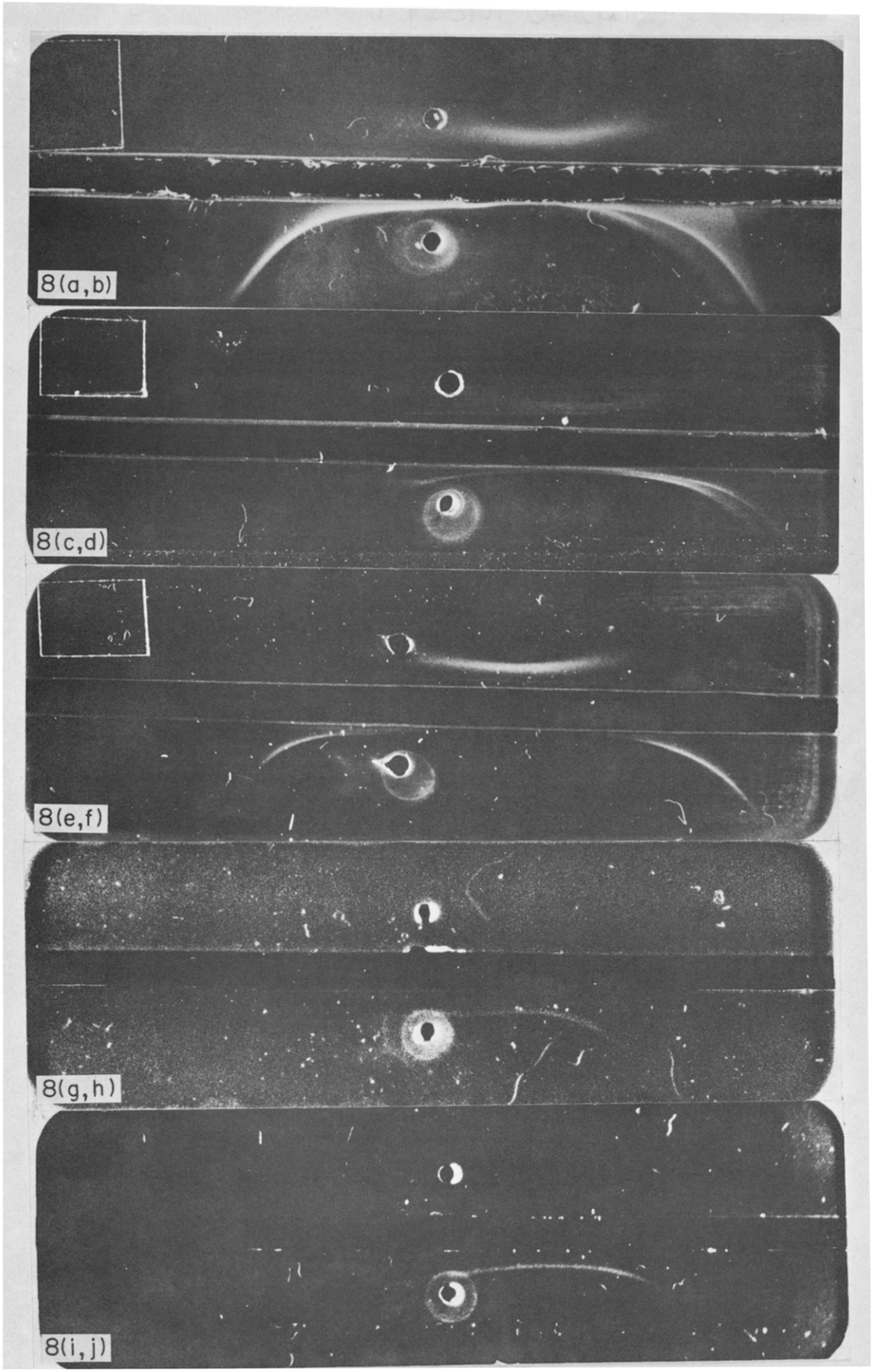

Fig. 8. IE of (a) (c) (e) (g) and (i) 4th peak fraction of lymphosarcoma serum and (b) (d) (f) (h) and (j) lymphosarcoma whole serum against (1) RAS IgG (H & L) (2) RAS L chain (3) RAS IgG (Fc) (4) RAS IgM (Fc) and (5) RAS IgA (Fc).

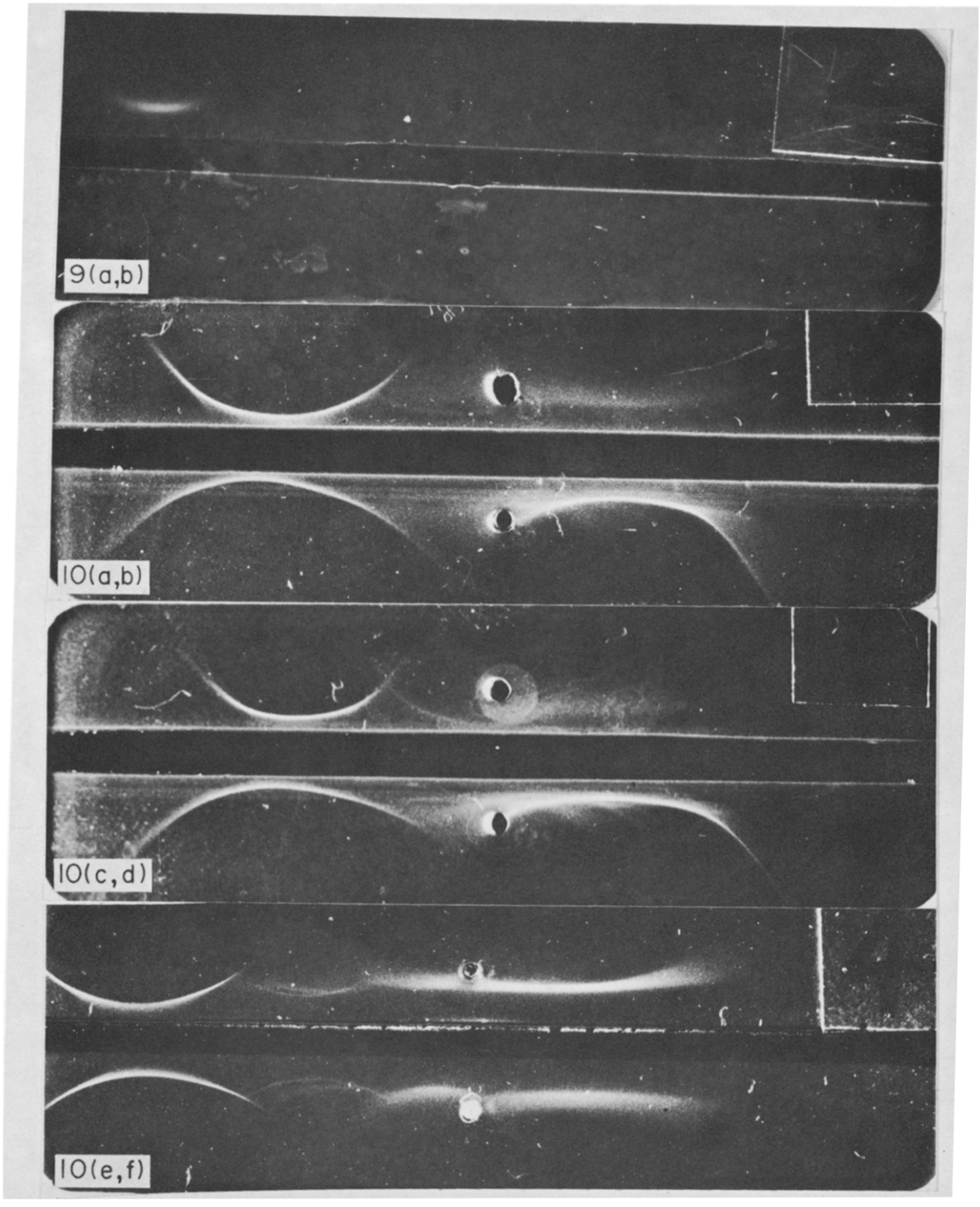

Fig. 9. IE of (a) 4th peak fraction from lymphosarcoma serum and (b) 4th peak fraction from normal pig serum against RAS albumin.

Fig. 10. IE of (a) urine (c) saliva (b) & (d) 4th peak fraction of serum from a lymphosarcoma pig and (e) & (f) lymph node suspension from a lymposarcoma and normal pig all against 4th peak fraction antibody in 1, 2 and 3.

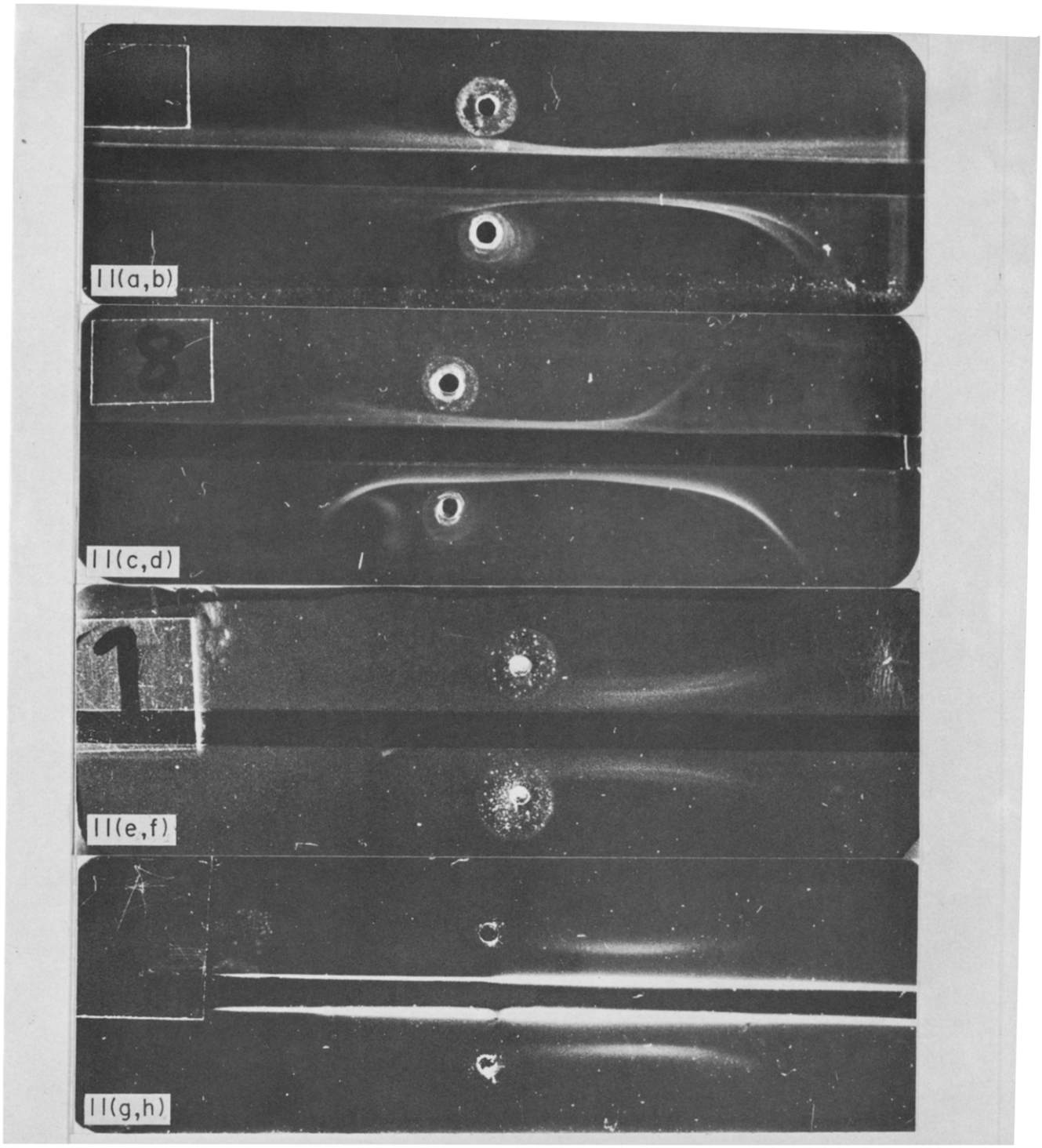

Fig. 11. *IE of* (*a*) & (*c*) *urine* (*b*) & (*d*) *serum* (*e*) & (*g*) *lymph node suspension all from a lymphosarcoma pig and* (*f*) & (*h*) *lymph node suspension from a normal pig against RAS light chain in* 1 *and* 3 *and RAS IgG* (*Fc*) *in* 2 *and* 4.

Fractionation of serum was on Sephadex G.200 using a 2.5 × 40 cm column with an upward flow adaptor and peristaltic pump giving a flow rate of 16–20 ml/hr using PBS at pH 7.2 with 0.02% sodium azide added. Monitoring was on an LKB Uvicord with an absorption of u.v. at 2537 Å and recording at 10 mm/hr on an LKB Chopper Bar Recorder.

Concentration of fractions was by vacuum dialysis using a Millipore-immersible molecular separator.

Antibody to 4th peak (see later) Sephadex G.200 fractions was produced in rabbits using Freund's incomplete adjuvant. Initially 0.5 ml (v/v) was given i.m., subsequent injections being given without adjuvant either i.m. or i.v. at periodic intervals over several months.

Specific antisera to pig IgG (Fc); IgM (Fc); IgA (Fc) IgG (H & L chains) and IgG (kappa and lambda light chain) were obtained from Nordic Immunological Laboratories Ltd.

Urine and saliva samples were collected from lymphosarcoma cases and normal litter mates. Saliva was obtained by injecting 1 ml of 2% (w/v) pilocarpine i.m. and aspirating from the buccal cavity. Mesenteric and gastro-splenic lymph node biopsies from lymphosarcoma and normal pigs were collected by laparotomy. Lymph nodes were macerated in a fine wire mesh sieve and made up in a concentration of 10 g lymph node/100 ml PBS, pH 7.2. Disruption of cells was completed by freezing and thawing. All debris was discarded after spinning at 3500 rev/min for 30 min. Storage of urine, saliva and lymph node suspension was similar to that for serum.

Fresh foetal pig sera were obtained from 70 day-old foetuses removed by hysterectomy from animals unrelated to the lymphosarcoma strain.

Molecular weight estimations were made on 5% acrylamide gel with 1% sodium dodecyl sulphate SDS added. Vertical electrophoresis was carried out in tubes using a Tris/glycine buffer, pH 8.3 with 1% SDS added. Samples were run at 8 v/cm for 1 hr and gels stained with 0.25% Coomassie Blue after fixing in 25% trichloroacetic acid for 30 min at 37°C and 7% acetic acid for 5 mins at 37°C. Gels were destained in a methanol, acetic acid water wash.

RESULTS

Total serum protein, albumin, alpha, beta and gamma globulin levels for 13 lymphosarcoma cases and a similar number of normal litter mates during a period from 8 to 30 weeks of age are shown in Table 1. From the 10th week the mean gamma globulin levels increased from 0.24 to 2.04 g/dl in the 24th week for lymphosarcoma cases. In contrast, the gamma globulin levels for normal litter mates increased from 0.12 to 0.47 g/dl for a similar period of time. Other differences can be seen in the albumin levels, which from the 14th week onwards remain consistently lower for the lymphosarcoma pigs, whereas the total serum proteins are higher in lymphosarcoma pigs from the 16th week onwards. from the 16th week onwards.

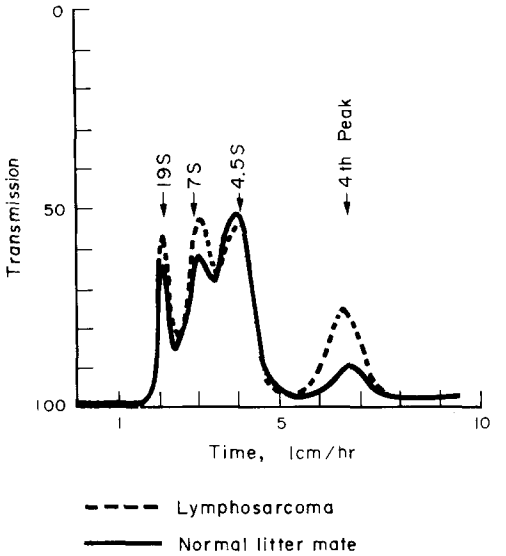

Fig. 1. Fractionation on Sephadex G200 of sera from a lymphosarcoma pig compared with a normal litter mate.

A comparison of serum protein levels was made in 14 lymphosarcoma pigs, and a similar number of randomly selected normal litter mates. Table 2 gives the results of *t*-tests

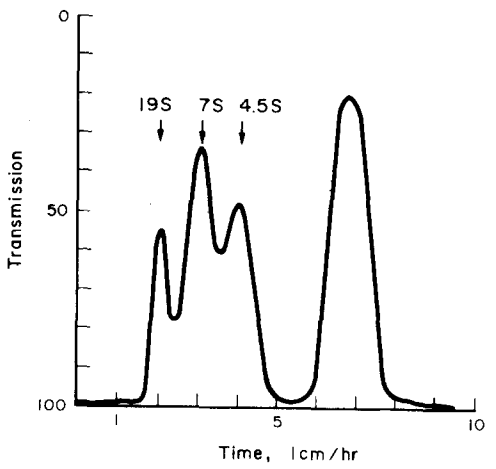

Fig. 3. Fractionation on Sephadex G200 of serum from a lymphosarcoma pig in the late stages of the disease.

on mean values for the two groups. Significant differences were found for albumin and gamma globulin levels ($P<0.01$). No significant differences were found between total proteins or alpha and beta globulin levels.

Separation of lymphosarcoma serum on Sephadex G.200 showed in comparison to serum from a normal litter mate, a larger 2nd and 4th peak, also a slightly increased 1st peak (Fig. 1). Electrophoresis of whole lymphosarcoma serum and isolated 2nd peak from a G.200 fractionation showed a polyclonal distribution in the IgG 2 region (Fig. 2). Immunoelectrophoresis (IE) of the 2nd peak on agar showed that when run against rabbit anti-pig IgG(H & L), IgM and IgA, it contained IgG and IgA, but no IgM. Subjecting the 2nd peak from the lymphosarcoma serum to ultracentrifugation on a sucrose gradient, revealed that the main molecules present were 7S.

In normal serum the 4th peak is small and regarded as being composed of low molecular weight products. The 4th peak in all lymphosarcoma serum was considerably increased as can been seen in Fig. 1, where the two traces from a normal litter mate and a lymphosarcoma case are compared. In some instances the 4th peak from a lymphosarcoma animal was found to be approximately one third of the total protein present (Fig.3). Antisera produced in rabbits against lymphosarcoma serum 4th peak were run on IE against 4th peak extracts. Several precipitin arcs with two predominant ones were found (Fig. 4). Of the two predominant ones, a major one can be located on the cathodic side of the insertion well in the heavy chain (Fc) region. The other predominant arc is in the albumin region and minor arcs occur in the $alpha_2$ and beta regions. Immunoelectrophoresis of normal and lymphosarcoma serum against 4th peak antiserum produced three arcs corresponding to IgM, IgA, and IgG, and other proteins corresponding to albumin and $alpha_2$ and beta globulins (Fig. 5). When foetal pig serum is run on IE against 4th peak antiserum, the albumin, $alpha_2$ and beta globulin arcs are present, but the main arc in the Fc region is absent (Fig. 6). Absorption of 4th peak antiserum with foetal pig sera removed all reaction to FPS, but left the Fc region antibody (Fig. 7). This antibody reacted with lymphosarcoma sera 4th peaks, but not normal litter mate sera 4th peaks. It also shows clear IgM, IgA and IgG lines of reaction when run against normal and lymphosarcoma whole sera.

These reactions suggested that 4th peak extracts from lymphosarcoma sera contain determinants for IgM, IgA and IgG, but no determinants are present in the 4th peak extracts from normal sera. To establish this possibility, lymphosarcoma 4th peak fractions and whole sera were run on IE against specific antisera, IgG (H & L); IgG (Light chain); IgG (Fc); IgM (Fc) and IgA (Fc). As can be seen from Fig. 8 reaction occurred between 4th peak fraction and all IgG antisera but not with IgM (Fc) nor IgA (Fc). This result indicates that lymphosarcoma 4th peak fractions contain light and heavy chain determinants for the IgG molecule and cross-reaction with IgA and IgM in whole sera as shown in Fig. 5 is due to common immunoglobulin light chain determinants. It further establishes that the 4th peak component corresponds to an incomplete IgG molecule, which includes both light and heavy chain determinants. This fact is emphasised by the reaction shown between 4th peak fraction and IgG (H & L); IgG (L) and IgG (Fc) antibodies which is similar to that observed between 4th peak fraction and 4th peak antiserum absorbed with FPS. (Fig. 7). Absorption of specific antisera IgG (Fc) and IgG (L) with 4th peak fraction from a lymphosarcoma serum removed all reaction against normal and lymphosarcoma sera and 4th peak fraction.

The presence of albumin in the 4th peak from lymphosarcoma cases was confirmed by IE of this fraction against specific rabbit anti-pig albumin antibody. (Fig. 9). Concentration of 4th peak fractions from normal litter mate sera did not reveal the presence of IgG light and heavy chain determinants, or foetal proteins, but a very faint trace of albumin could be detected, although this is not seen easily in Fig. 9.

Urine and saliva from lymphosarcoma cases and saline extracts of macerated mesenteric lymph nodes taken from affected and normal animals were examined for the presence of the 4th peak components. As can be seen in Fig. 10, urine and saliva from affected animals and lymph node suspension from both normal and affected animals all reacted with 4th peak antibody. The reaction with saliva shows an albumin and beta globulin arc, whereas urine and lymph node extracts show reactions similar to that seen in Fig. 4 between 4th peak fraction and 4th peak antibody. Urine and lymph node extracts also react with IgG (Fc) and IgG (L) antisera as shown in Fig. 11. Again the reaction seen in Fig. 11 is identical

to that shown in Fig. 7, between 4th peak antibody absorbed with FPS and 4th peak fractions from lymphosarcoma sera. Absorption of IgG (Fc) and IgG (L) with 4th peak fraction from a lymphosarcoma case removed all reaction to urine and lymph node suspension. A similar exercise using 4th peak fraction from a normal animal did not remove this reaction.

The molecular size of the 4th peak incomplete IgG molecule was estimated on SDS acrylamide using ovalbumin and bovine serum albumin as markers. Migration distances measured indicate that the main component seen in the 4th peak has a molecular size in the region of 50 to 60 $\times 10^3$.

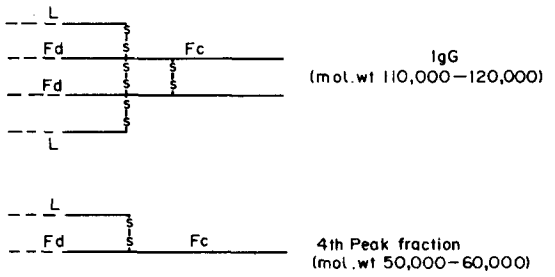

Fig. 12. Suggested structure of the incomplete IgG molecule containing both heavy and light chain determinants obtained from the 4th peak of a Sephadex G200 fractionation.

DISCUSSION

Since the first case of heavy chain disease (γ-HCD) was reported in man by Franklin *et al.* [7] approximately 35 cases have been reported in the literature. Following this initial case, two other types of HCD have been recognised in man corresponding to the class of immunoglobulin heavy chain involved. The α HCD-type was first reported by Seligman *et al.* [8] and then the μHCD-type by Forte *et al.* [9]. To date, nothing comparable has been seen in animals other than man. There have been deletions in the immunoglobulin molecules produced by myeloma tumour cell lines from the mouse [10, 11], but nothing resembling HCD has been seen in other animals. Multiple myeloma with paraproteins in the plasma and Bence–Jones proteins in the urine have been described for various species of animal such as the horse [12]; cow [13]; dog [14–18]; cat [19–21]; rabbit [22] and mouse [23, 24]. In none of these cases has the presence of a deleted IgG molecule been observed.

Although numerous cases of sporadic lymphosarcoma have been described in the pig [25–29], no immunoglobulin fragments in the plasma or urine have been recorded.

In hereditary lymphosarcoma, an IgG heavy chain (γFc) and light chain (γL) component has been identified in the serum and urine. This is a constant feature in all cases observed, especially in the later stages of the disease. These components appear to come from extracts of lymphosarcomatous mesenteric lymph nodes. They are also present in normal mesenteric lymph nodes, but cannot be found in normal serum or urine. It would seem, therefore, that these components do not appear to be exclusively associated with tumour cells, but could be associated with the type of lymphocyte from which the tumour cell is derived. The stage at which tumour cells become active, or alternatively, when they die may be a contributing factor. Conversely, the reaction of unrelated cell types, which could be stimulated in the presence of tumour cells may be another source of these components. In either case the presence of foetal cell products indicates an immature phase of development in the cells involved. Histopathology of the cell types seen in affected lymph nodes indicate that initially there is a preponderance of lymphoid cells which changes to a mixed cellularity and terminates with fibroreticular growth and lymphocellular depletion. Numerous macrophages, eosinophils and plasma cells are evident in the terminal phase [3]. The exact time when the γ Fc and γ L component appears in the serum and urine has yet to be determined. An obvious increase in gamma globulin levels occurs at the 16th week of age and this could be consistent with the increase in complete molecules of IgM and IgG.

The observation that similar γFc and γL fragments can be found in normal mesenteric lymph nodes would seem to confirm some of the observations made in man. It is known that free immunoglobulin light chains can be found in normal human plasma [30, 31], also in normal urine [32], and Fc γ-like proteins have been detected in normal plasma [33,34]. Bienenstock [35], demonstrated that urine from normal healthy subjects can contain γG heavy chain fragments similar to Fc and another component called F'c which could be obtained from Fc fragment by digestion with esterase. It has also been shown by Turner and Rowe [36], that normal urine contains material resembling F'c fragment of IgG. This was released from IgG by digestion with papain or trypsin and was smaller than Fc. The absence of γFc and γL fragments in the

serum and urine of normal pigs may merely indicate a lack of reaction within the lymph node concerned. In hereditary lymphosarcoma cells may be activated at a stage of development when the assembly of the immunoglobulin molecule is incomplete. The only case recorded in man where free γFc and γL chains were detected together in the serum and the urine was a lymphoma-like condition reported by Isobe and Osserman [37]. In some respects this case shows certain similarities to the condition described here. The main difference however, involves the molecular size of the main component, which is approximately half the size of a normal IgG molecule. A schematic representation of this component could be as shown in Fig. 12, with the light and heavy chains linked to form half an IgG molecule. This form of the immunoglobulin molecule has not been suggested before, but would give a reason for the absence of detectable free γL chains in the serum and urine. There is no theoretical reason why the binding of one light and one heavy chain as shown could not take place before complete assembly of the molecule.

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