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The Relative Proportions of IgG-, IgA- and IgM-containing Cells in Rabbit Tissues during Experimental Trichinosis

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Summary. The relative proportions of IgG, IgM and IgA immunoglobulin-containing cells were determined in the intestinal mucosa, spleen, popliteal lymph nodes and diaphragm of rabbits after a single infection and after hyperinfection with *Trichinella spiralis*. Staining with pairs of immunofluorescent reagents, specifically reactive with γ, μ or α immunoglobulin heavy chains and labelled with contrasting fluorochromes, permitted direct counting of cells containing two different immunoglobulin classes in a single tissue section. By employing two different pairs of reagents on adjacent sections the relative numbers of cells containing IgG, IgM and IgA were calculated.

The observed cellular distribution of γ, μ and α heavy chains in the rabbit intestinal mucosa corresponded with the reported distribution in the human intestine. A relative increase in IgM-containing cells in the mucosa was observed after early infection with *Trichinella*, followed by an apparent increase in cells with IgG late in infection and after hyperinfection. The proportion of cells staining for IgA remained uniformly high in the intestine throughout the course of infection.

The proportions of cells containing different immunoglobulin classes in the spleen contrasted with those observed in the intestinal mucosa, particularly with respect to cells containing α chain. IgA cells made up 2–10 per cent of the immunoglobulin-containing cells in the spleen as compared to 80–90 per cent in the intestinal mucosa. Most spleen sections showed an increase in IgM cells late in infection with *Trichinella* and after hyperinfection. The proportions of immunoglobulin-containing cells in the popliteal lymph nodes generally paralleled those observed in the spleen.

Local cellular infiltration of the diaphragm occurred at the time of larval encystment. Immunoglobulin-containing cells were often prominent and the cellular distribution of immunoglobulin classes resembled that found in the spleen.

The indirect fluorescent antibody technique was employed to detect anti-*Trichinella* antibody of the three immunoglobulin classes in sera and extracts of the gut of hyperinfected rabbits. Only IgG antibody was detected in gut extracts although both IgG and IgA were demonstrated to be present by Ouchterlony analysis. Both IgG and IgA were demonstrated to be present by Ouchterlony analysis. Both IgG and IgM antibodies were demonstrated in the sera.

INTRODUCTION

The immunoglobulin classes IgG, IgM and IgA can be distinguished by antigenic differences in their heavy polypeptide chains γ, μ and α, respectively* (Cohen and Porter,

* The nomenclature used for the classes of immunoglobulins and polypeptide chains is that published in *Bull. Wld Hlth Org.* (1964) **30**, 447.

1964). Recently immunofluorescent reagents have been prepared which react specifically with either γ, μ or α rabbit immunoglobulin heavy chains. In addition, technical improvements in preparation of fluorescent antibody reagents has allowed staining by pairs of reagents labelled with contrasting fluorochromes and facilitated the direct determination of the cellular distribution of heavy chains in rabbit lymphoid tissues. (Cebra and Goldstein, 1965; Cebra, Colberg and Dray, 1966). A high degree of lymphoid cell differentiation with respect to content of immunoglobulin class has been established (Cebra *et al.*, 1966; Mellors and Korngold, 1963); thus staining with pairs of contrastingly labelled anti-heavy chain reagents offers a convenient technique for determining the relative numbers of cells containing IgG, IgM and IgA immunoglobulin.

Recently, differential changes in serum concentrations of the various immunoglobulin classes in a number of diverse, human diseases has been tabulated (McKelvey and Fahey, 1965). It was considered of interest to determine if relative changes in the numbers of lymphoid cells containing different classes of immunoglobulin could be detected during the course of an experimental infection. The present investigation was initiated to study the cellular distribution of immunoglobulins before and during experimental trichinosis.

Infection with the helminth parasite, *Trichinella spiralis*, has been extensively studied and is a well characterized experimental infection in which antibody response and acquired resistance can be readily demonstrated (Gould, 1945; Kagan, 1960). Although it is clear that specific antibody and local cellular response (hypersensitivity) are significant factors in acquired resistance, the types of antibody involved and the role of local tissue immunity or antibody production are still uncertain (Larsh, 1963). The purpose of this study was to determine the relative proportions of cells containing IgG, IgM and IgA in those tissues which might be of significance in local as well as general antibody response to infection in singly exposed and hyperinfected rabbits. The tissues studied were the upper intestine, the site of initial infection and immunological response to reinfection; the spleen and lymph nodes, the sites of antibody production to circulating antigens; and skeletal muscle (diaphragm), the site of larval growth and encystment.

MATERIALS AND METHODS

Fluorescent antibody reagents

The preparation and fluorochrome labelling of anti-μ, anti-α and anti-γ rabbit heavy chain reagents and the optimal proportions of these reagents in mixtures of any two labelled with contrasting fluorochromes has been published (Cebra *et al.*, 1966; Cebra and Goldstein, 1965).

In this study, the following pairs of immunofluorescent reagents were employed: tetramethylrhodamine isothiocyanate labelled (red) anti-γ chain with fluorescein isothiocyanate labelled (green) anti-μ chain; red anti-α chain with green anti-μ chain; and red anti-γ chain with green anti-α chain.

Animals

Ten young, adult New Zealand rabbits of either sex, weighing 5–8 lb, were used. All were inoculated *per os* with 10,000 *Trichinella* muscle larvae. Six were killed at intervals after an initial infection and four animals were reinfected with 12,000 larvae, 1 month after

the first infection, and killed at intervals after hyperinfection. Time intervals from infection to necropsy are listed in Table 1. Inoculation of larvae was done by a previously described method (Crandall, 1965).

TABLE 1

DISTRIBUTION OF γ, μ AND α IMMUNOGLOBULIN HEAVY CHAINS AMONG LYMPHOID CELLS IN THE DUODENAL MUCOSA DURING EXPERIMENTAL TRICHINOSIS

Rabbit No.	Treatment† days‡	Percentage of cells stained by each fluorochrome with the following pairs of antibody reagents								
		Red anti-γ green anti-μ			Red anti-α green anti-μ			Red anti-γ green anti-α		
		γ (%)	μ (%)	Cells counted	α (%)	μ (%)	Cells counted	γ (%)	α (%)	Cells counted
1	*Trichinella* infected 7 days	61	39	307	95	5	476			
		65	35	432						
2	Infected 10 days	60	40	634	92	8	535	10	90	334
		63	37	204						
	P	64	36	294	89	11	298			
3	Infected 12 days	61	39	396	94	6	436			
		69	31	465	96	4	625			
	P	67	33	629	96	4	603			
4	Infected 12 days	66	34	232	96	4	807	9	91	547
5	Infected 20 days	95	5	323	98	2	455			
		95	5	358	98	2	632			
6	Infected 31 days	95	5	231	99	1	630			
7	*Trichinella* hyperinfected 7 days	91	9	304	99	1	275			
8	Hyperinfected 8 days	96	4	801	99	1	999	32	68	571
9	Hyperinfected 9 days	97	3	581	99	1	560	11	89	329
		97	3	343						
		93	7	239	98	2	590			
10	Hyperinfected 12 days	92	8	210	98	2	121			
11	Uninfected	99	1	132	99	1	1326	4	96	180
12	Uninfected	89	11	220	99	1	494			
		90	10	105						
13	Uninfected	92	8	349				6	94	173
	P	89	11	171						
14	Uninfected	80	20	188	97	3	156			
		81	19	214	98	2	322			
	P	91	9	160	96	4	357			
15	Antigen injected: *Salmonella*	92	8	617	98	2	1107	20	80	498
		87	13	58	99	1	293			
16	Antigen injected: *Trichinella*	92	8	147	98	2	502			
17	Antigen injected: rat γ-globulin	95	5	293	98	2	329			

P, Counts from sections of ileum.

† Treatment: Rabbits 1–10 infected with 10,000 *Trichinella* larvae; Rabbits 7–10 reinfected at 30 days with 12,000 larvae.

‡ Days indicate time from infection to necropsy.

Four uninfected rabbits were used as controls; two were similar in age and weight to the infected animals; a third, although similar in age, appeared emaciated and unhealthy; a fourth was an animal heterozygous (Aa^1/Aa^2) at the *a* locus, which controls allotypic markers on heavy chains, and was of unknown history.

A group of three rabbits had been utilized in other studies for antibody production. They were over 6 months old and weighed more than 10 lb. They had received multiple injections of heat-killed *Salmonella*, rat γ-globulin or saline extract of *Trichinella* larvae, and they had demonstrable antibodies to the injected antigen at the time of necropsy.

Tissue preparation and staining

Immediately after killing the animals a small segment of the duodenum was removed, cut into small pieces and frozen with carbon dioxide on to microtome chucks; tissue samples were also taken from the spleen of most animals. In selected animals, additional tissue samples were taken from the ileum, the diaphragm, and popliteal lymph nodes. Sections were cut at 5–7 μ in a cryostat, fixed in 95 per cent ethanol for 15 minutes and air dried. Sections were stained for 1 hour with the immunofluorescent reagents, washed with phosphate buffered saline (pH 7·4) and mounted in buffered glycerine. Adjacent cryostat sections from each tissue were fixed and stained with haematoxylin and eosin by routine procedures. Sections stained with the pairs of immunofluorescent reagents were examined under a Leitz fluorescence microscope with a Corning No. 5840 excitor filter and K2,23A or 57A eyepiece filters. The use of these ocular filters to detect double staining of single cells has been described (Cebra and Goldstein, 1965). In the present study, only rarely was a doubly stained cell observed and the K2 filter was used almost exclusively. Sections were scanned systematically after staining and cell counts made. The results were expressed as the percentage of cells staining with each of the contrasting fluorochromes.

Demonstration of immunoglobulin and antibody

Preparations from rabbit intestines were made for testing immunoglobulin and antibody content of this tissue. The whole small intestine was placed in cold saline, cut longitudinally and the contents washed out with 100–200 ml of phosphate buffered saline (pH 7·4). The intestine was divided into anterior and posterior halves and each half was homogenized in a blender with 100 ml of physiological saline. The intestinal content and wall homogenates were centrifuged at 8000 ***g*** for 10 minutes and the supernate was dialysed with the aid of pressure at 4° against buffered saline to approximately one-fifth the original volume. In several cases, protein was precipitated from the supernatant with half-saturated ammonium sulphate and the washed precipitate was redissolved in buffered saline. These preparations, as well as sera collected from the rabbits at necropsy, were tested for the presence of the three immunoglobulin classes by Ouchterlony analysis employing monospecific unlabelled anti-heavy chain reagents (Cebra and Robbins, 1966).

Tests for *Trichinella* antibody in the three immunoglobulin classes were carried out by the indirect fluorescent antibody technique. Cryostat sections of rabbit diaphragm containing mature larvae were used to detect antibody in intestinal preparations and in serum reactive with *Trichinella*. Diaphragm sections flooded with the test preparations (intestinal homogenates, undiluted or diluted with saline 1:2; serum, undiluted or diluted 1:5) were kept in a moist chamber for 30 minutes at room temperature, washed thoroughly with buffered saline and stained for 30 minutes with fluorochrome-labelled anti-μ, anti-γ or anti-α heavy chain reagent. Control sections were treated with identical preparations from

uninfected animals and other control sections were treated with saline prior to immunofluorescent staining.

RESULTS

IMMUNOHISTOLOGICAL EXAMINATION OF TISSUES

Intestine of normal rabbits

Staining with pairs of immunofluorescent reagents revealed numerous brightly stained mononuclear cells in the lamina propria of the intestinal mucosa which were clearly differentiated by colour. Immunoglobulin-containing cells were not usually seen in the submucosa of uninfected animals. There was little background staining; anti-γ reagent produced some diffuse interstitial staining and anti-α reagent stained the proximal portion of the glandular crypt cells slightly, but this staining did not interfere with recognition of the intense intracellular staining of lymphoid cells. Whitish-yellow fluorescent cells with granular cytoplasm, probably eosinophils, were present in the intestinal mucosa in varying numbers, but these were usually easily distinguished by morphology and colour from the specifically stained lymphoid cells.

In all intestinal sections examined there was a preponderance of cells containing α chain. The high proportion of these cells compared with cells containing μ and γ chains is shown in Table 1. A calculation of the percentage of cells containing each class of immunoglobulin using the γ:μ and μ:α percentages indicated that cells with α chain make up 80–90 per cent of the immunoglobulin-containing cells, with few exceptions.

The α chain-containing cells were prominent in the lamina propria of the villi, often appearing as compact strands or groups which extended into the core of the villus often without distinct cell outlines (Figs. 1 and 2). These cells were also numerous in the connective tissue between the crypts of Lieberkuhn in the lower lamina propria. In contrast, cells staining for γ and μ chains were usually found scattered in the lamina propria and were more concentrated in the basal areas near the muscularis mucosa than in the villi (Figs. 4 and 5). This general relationship appeared to hold true for the intestinal mucosa of both the duodenum and the ileum.

The lymphoid cells staining for heavy chain appeared somewhat variable in size and in nuclear–cytoplasmic ratio. There was no obvious correlation between cell morphology and class of heavy chain in the cytoplasm. Haematoxylin and eosin staining of adjacent sections demonstrated plasma cells, as well as other lymphoid cells (large and small lymphocytes) in those areas containing numerous cells which fluoresced after staining with anti-heavy chain reagents (Figs. 3 and 6).

Intestine of rabbits infected with Trichinella

Cryostat sections of the anterior intestine of animals infected with *Trichinella*, when stained with haematoxylin and eosin, showed an increased infiltration of the lamina propria with cells. This increased cellularity was more evident in hyperinfected animals. The cellular infiltrate was primarily mononuclear in the singly infected rabbits. Granulocytes which were mainly pseudo-eosinophils were prominent only in certain sections from hyperinfected animals, where they were found both in the mucosa and upper submucosa. In a few sections from both singly infected and hyperinfected rabbits, adult *Trichinella* were observed in the mucosa but there was no particular cellular response to the helminths.

Immunofluorescent staining of intestinal sections from rabbits (Nos. 1–4) studied soon after *Trichinella* infection (days 7–12) revealed a relative increase in μ-chain-containing cells (Table 1). In these sections IgM cells made up at least one-third of the total IgM plus IgG population. Calculation of the relative percentages of cells containing each class of immunoglobulin indicated that μ cells ranged from 4 to 7 per cent of the total in these sections, as compared to 1–3 per cent in all other intestinal sections studied. The calculated percentage of cells containing γ chain ranged from 7 to 11 per cent, with a mean between 8 and 9 per cent. This mean was identical with the mean percentage of γ-cells in the uninfected animals calculated from a similar range of 6–11 per cent IgG cells.

In rabbits 5 and 6 and hyperinfected rabbits (Nos. 7–10) the relative number of cells stained for μ chain in the intestinal mucosa was consistently lower than that in the rabbits killed earlier and there was an apparent increase in the relative numbers of cells stained for γ chain. A calculated percentage of cells staining with anti-γ chain ranged from 10 to 27 per cent with a mean of 19 per cent.

An overall comparison of the relative numbers of cells containing different classes of heavy chain in the intestinal mucosa among hyperinfected animals, uninfected animals and antigen stimulated animals did not show any marked differences which might be considered significant, although uninfected animals had a somewhat lower relative number of γ chain cells, as mentioned previously. The proportion of cells containing α chain was consistently high in all intestinal sections and this high proportion may have led to some counting errors, which are discussed later.

Spleen and popliteal lymph nodes

The cell counts from doubly stained spleen and popliteal lymph node sections are summarized in Table 2. The calculated proportions of fluorescing cells in spleen sections containing different heavy chain classes were 2–10 per cent α chain, 27–69 per cent γ.

During *Trichinella* infection no changes in the relative proportion of cells containing α chain were apparent. In spleen sections from animals killed soon after infection (Nos. 1, 2 and 3), there was a relatively high percentage of IgG cells in relation to IgM cells. In late infection (No. 6) and in hyperinfected animals, the number of immunoglobulin-containing cells was obviously increased and the percentage of IgM and IgG cells was reversed, with one exception (No. 8). Sections from one hyperinfected animal (No. 10) revealed many

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FIGS. 1–6. Cryostat sections of rabbit duodenum stained with pairs of fluorochrome-labelled anti-heavy chain reagents or haematoxylin and eosin. Sections are from rabbits that were uninfected or in early *Trichinella* infection.

FIG. 1. Intestinal villus stained with rhodamine labelled anti-α chain and fluorescein labelled anti-γ chain. Photographed through 23A barrier filter to show only rhodamine-stained cells (γA-immunoglobulin). ×266.

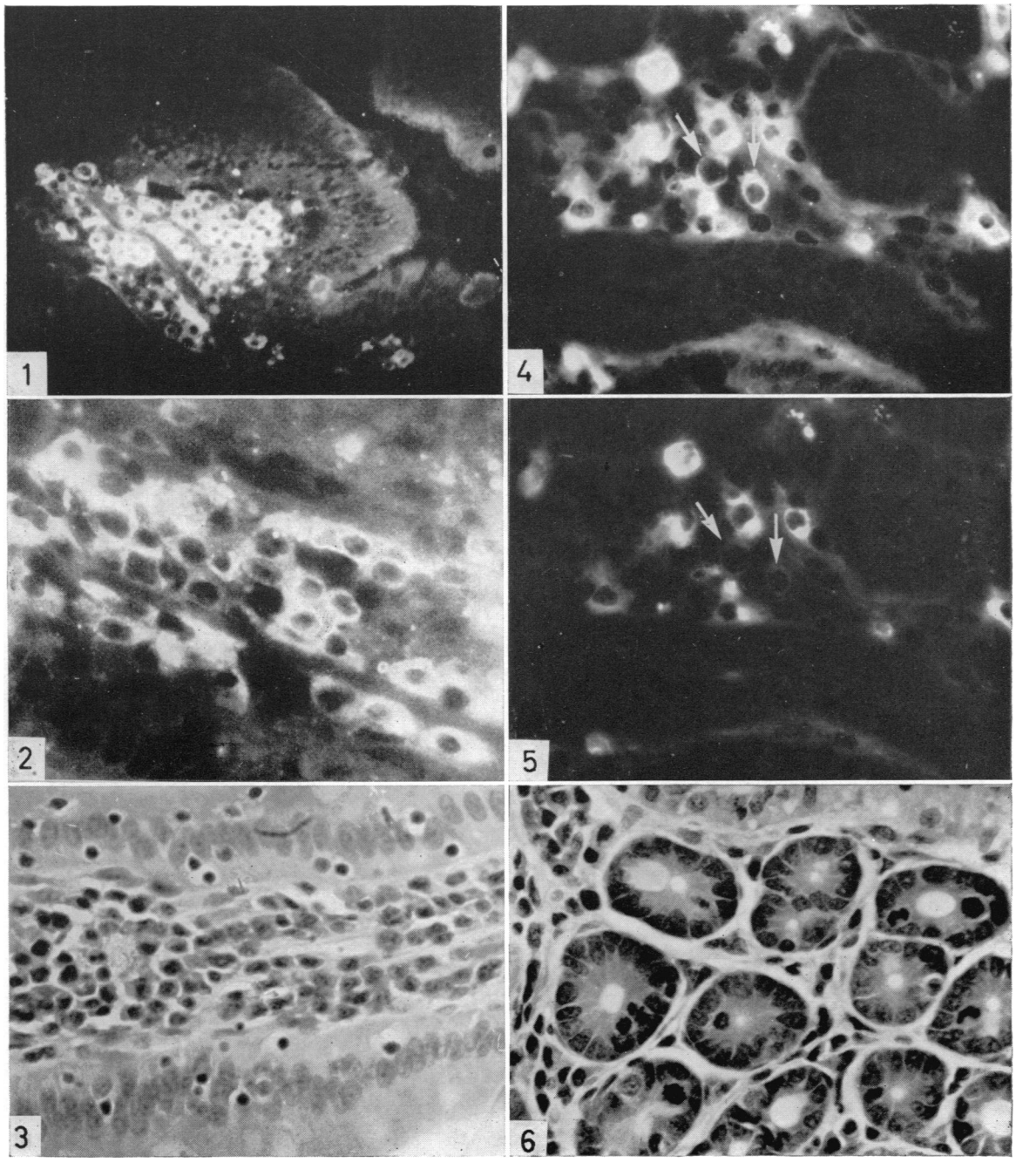
FIG. 2. Intestinal villus stained as in Fig. 1. Stained cells in core of villus contain α immunoglobulin heavy chain. ×633.

FIG. 3. Intestinal villus stained with haematoxylin and eosin showing distribution of lymphoid cells in lamina propria. A moderate degree of lymphoid cell infiltration is evident. ×400.

FIG. 4. Basal lamina propria of intestine stained with fluorescein labelled anti-μ chain and rhodamine-labelled anti-γ chain. Photographed through K2 filter to show both IgM and IgG-containing cells. Arrows mark two cells staining for μ chain. ×432.

FIG. 5. Same field as in Fig. 4 photographed through 23A barrier filter to show only IgG-containing cells. ×432.

FIG. 6. Basal lamina propria of intestine stained with haematoxylin and eosin showing distribution of lymphoid cells between glandular crypts. ×400.



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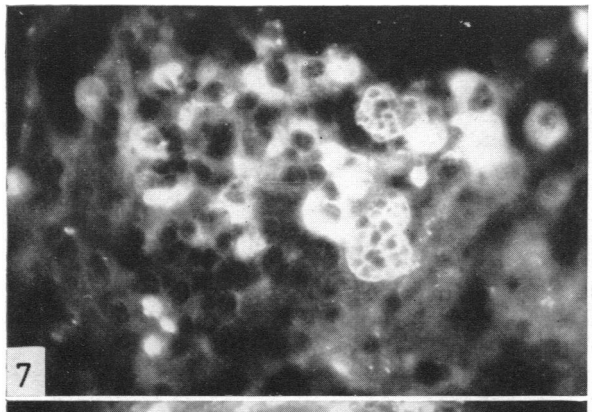
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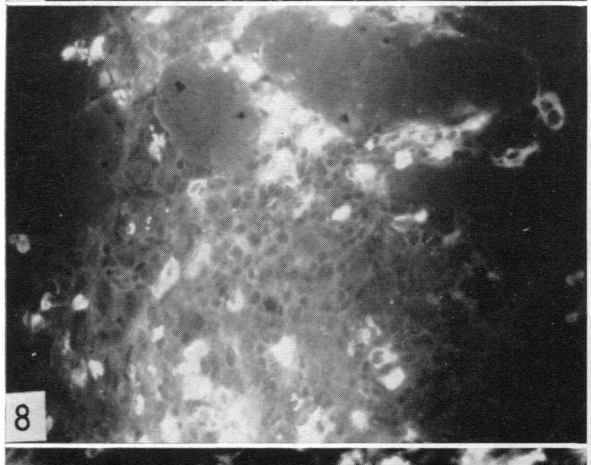
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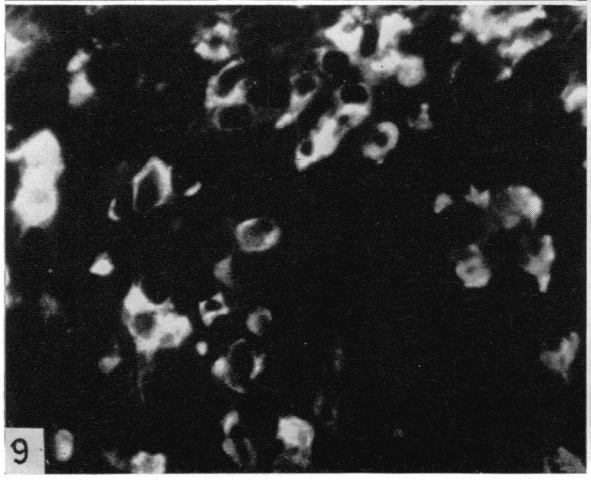
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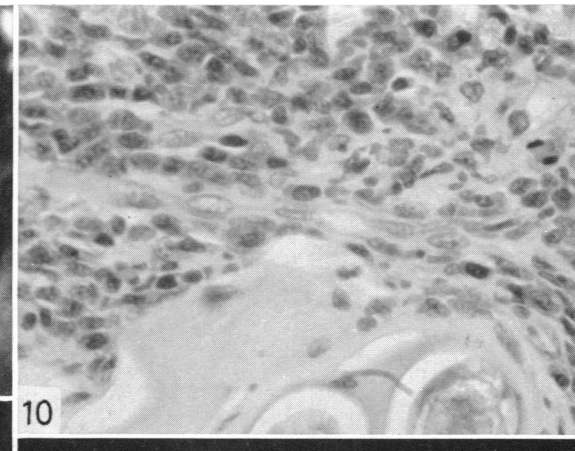
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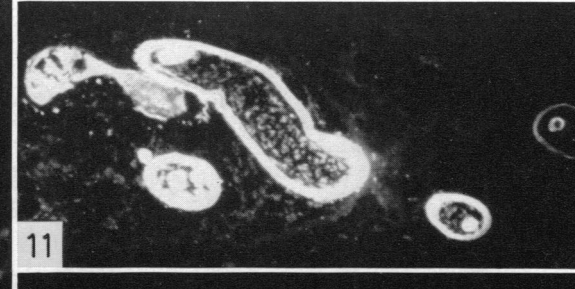
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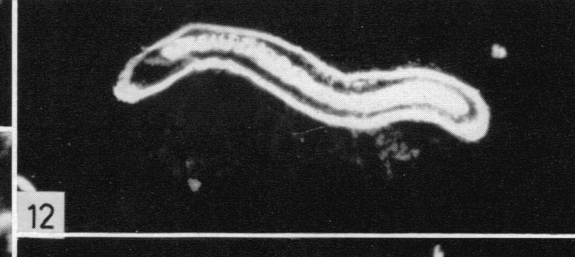
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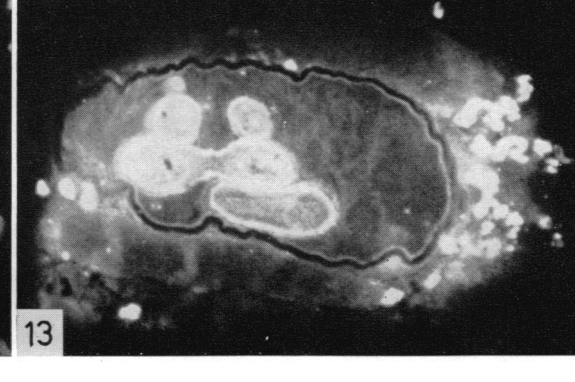
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TABLE 2

DISTRIBUTION OF γ, μ AND α IMMUNOGLOBULIN HEAVY CHAINS AMONG LYMPHOID CELLS IN SPLEEN AND POPLITEAL LYMPH NODES DURING EXPERIMENTAL TRICHINOSIS

Rabbit No.	Treatment		Antibody reagent pairs: Red anti-γ green anti-μ γ (%)	μ (%)	Cells counted	Red anti-α green anti-μ α (%)	μ (%)	Cells counted	Red anti-γ green anti-α γ (%)	α (%)	Cells counted
1	*Trichinella* infected 7 days	sp	68	32	441	9	91	111			
2	Infected 10 days	sp	75	25	139	9	91	59	94	6	137
		pl	63	37	222	16	84	118	80	20	150
3	Infected 12 days	sp	61	39	137	16	84	99			
6	Infected 31 days	sp	42	58	142	12	88	188			
7	Hyperinfected 7 days	sp	44	56	131	7	93	162			
8	Hyperinfected 8 days	sp	58	42	406	19	81	123	91	9	532
		pl	54	46	427				94	6	376
9	Hyperinfected 9 days	sp	38	62	227	16	84	187	86	14	110
		pl	32	66	713	13	87	286	82	18	125
10	Hyperinfected 12 days	sp	39	61	248						
11	Uninfected	sp	74	26	201	24	76	36	89	11	155
		pl	72	28	309				90	10	360
12	Uninfected	sp	66	34	119	18	82	51			
		pl	61	39	196	7	93	53			
13	Uninfected	sp	30	70	111	14	86	129			
		pl	45	55	289				89	11	241
14	Uninfected	sp	51	49	509	9	91	248			
15	Antigen: *Salmonella*	sp	53	47	53	25	75	151			
		sp	56	44	160	23	77	129			
		pl	36	64	927	7	93	183	96	4	534
16	Antigen: *Trichinella*	sp	57	43	299	5	95	256			
17	Antigen: rat γ-globulin	sp	47	53	260	9	91	109			

sp, Spleen sections; pl, popliteal lymph nodes sections.

FIG. 7. Spleen section from *Trichinella* infected rabbit (No. 10) stained with fluorescein-labelled anti-μ chain and rhodamine-labelled anti-γ chain. The two conspicuous vesicular cells, 'grape cells' or 'Russell body' cells, are stained for μ chain. × 400.

FIG. 8. Diaphragm section from *Trichinella* infected rabbit (No. 7) stained with fluorescein-labelled anti-μ chain and rhodamine-labelled anti-γ chain. Majority of fluorescent cells are stained for μ chain. × 266.

FIG. 9. Diaphragm section from *Trichinella* infected rabbit (No. 7) stained as in Fig. 8. × 633.

FIG. 10. Diaphragm section from *Trichinella* infected rabbit (No. 9) stained with haematoxylin and eosin. Cellular infiltrate around cyst contains lymphoid cells. × 400.

FIG. 11. *Trichinella* larva in diaphragm section stained by indirect method with intestinal extract (No. 8) and rhodamine-labelled anti-γ chain. × 300.

FIG. 12. *Trichinella* larva in same section as Fig. 11. Staining of the intestinal tract of the larva is shown. × 300.

FIG. 13. *Trichinella* larva in diaphragm section stained by indirect method with macroglobulin fraction of serum (No. 8) and rhodamine-labelled anti-μ chain. Fluorescent deposits near outer edge of cyst are largely non-specific. × 300.

cells staining with anti-μ chain reagent which had a brightly fluorescent alveolar cytoplasm (Fig. 7). These cells resembled the plasma cells with 'Russell bodies' or 'grape' cells pictured by Mellors and Korngold (1963)

Uninfected animals presented a varied picture. Rabbits similar to those utilized for experimental *Trichinella* infection (Nos. 11 and 12) had spleen ratios of IgG to IgM cells similar to those in early *Trichinella* infection. Spleen sections from rabbit 13 revealed numerous immunoglobulin containing cells with a predominance of IgM cells. Rabbit 14, which was obviously unhealthy and had a grossly enlarged spleen, revealed an almost equal proportion of IgM and IgG cells. The animals injected with antigens had a percentage of IgM cells nearly equal or slightly higher than the percentage of IgG cells.

In sections of popliteal lymph nodes, the relative proportions of cells containing different classes of immunoglobulin heavy chain paralleled the proportions found in the spleens. One exception was rabbit 15, in which a higher proportion of IgM cells was apparent in the popliteal lymph nodes. Perhaps of some significance was the fact that this rabbit had received antigen injections in the footpads.

Diaphragm

The changes were typical of *Trichinella* infection (Gould, 1945). Diaphragm sections from rabbits infected for 10 and 12 days contained small, immature larvae within the muscle fibres. The infected fibres were undergoing obvious granular degeneration, but there was little inflammatory cell response. At 21 days of infection (No. 5) extensive

TABLE 3

DISTRIBUTION OF γ, μ AND α IMMUNOGLOBULIN HEAVY CHAINS AMONG LYMPHOID CELLS IN THE DIAPHRAGM OF *Trichinella* INFECTED RABBITS

Rabbit No.	Treatment	Antibody reagent pairs								
		Red anti-γ green anti-μ			Red anti-α green anti-μ			Red anti-γ green anti-α		
		γ (%)	μ (%)	Cells counted	α (%)	μ (%)	Cells counted	γ (%)	α (%)	Cells counted
6	*Trichinella* 31 days	44	56	272	44	56	54			
7	*Trichinella* hyperinfected 7 days	39	61	176	28	72	146			
9	*Trichinella* hyperinfected 9 days	39	61	353	21	79	200	57	43	180

muscle degeneration was apparent; most larvae were large, coiled and surrounded by a dense homogeneous matrix of degenerated muscle. No marked cell infiltration was present except in a few scattered foci and fluorescent staining with anti-immunoglobulin heavy chain reagents showed only an occasional stained cell.

Sections of diaphragm from rabbit No. 6 infected for 31 days, and from hyperinfected rabbit No. 7 showed obvious cyst wall formation surrounding the larvae and a conspicuous cellular infiltration. This cellular infiltrate was of mixed character: granulocytes, histiocytes and mononuclear lymphoid cells, some of which were typical plasma cells. Immunofluorescent staining revealed cells containing each class of heavy chain, with IgM cells the most abundant class (Table 3) (Figs. 8 and 9). Diaphragm sections of hyperinfected

rabbits 9 and 10, necropsied 9 and 10 days after reinfection, revealed a more extensive myositis than the previous sections, but the cellular distribution of immunoglobulins and the cellular components of the infiltrate were generally similar (Fig. 10). Only a few areas in the diaphragm sections of rabbit No. 10 were suitable for accurate counting of stained cells; however, many cells stained for μ-chain were present which had the 'grape' cell appearance of the cells found in the spleen of this same animal.

IMMUNOGLOBULIN AND ANTIBODY TO *Trichinella* IN TISSUE EXTRACTS

Ouchterlony analysis of gut extracts and contents from infected, hyperinfected and normal rabbits demonstrated the presence of γ- and α-heavy chains, presumably in IgG and IgA. In every case, the precipitin lines formed by gut extracts were stronger than those given by intestinal contents. The μ chain was not detected in any of the gut preparations. In contrast sera from these same animals gave precipitin lines with anti-μ and anti-γ chain reagents but faint or no lines with anti-α chain.

Indirect staining of *Trichinella* larvae in diaphragm sections to detect antibody of the different immunoglobulin classes was carried out with anterior gut extracts from hyperinfected rabbits (Nos. 7, 8 and 9). After treatment with both undiluted and diluted extracts, bright specific staining of larvae was observed only with anti-γ chain reagent. Localization of fluorescence was predominently on the cuticle (and body wall in some cases), and gut of the larvae (Figs. 11 and 12). Sera from the hyperinfected animals gave similar staining of larvae with anti-γ chain and faint but positive staining with anti-μ chain. Sera from two animals killed 10 and 12 days after a single infection gave only a questionable staining with anti-γ reagent and no staining with the other anti-heavy chain reagents.

To confirm the presence of *Trichinella* antibody in the serum macroglobulins, a Sephadex G-200 column fractionation of serum from hyperinfected rabbit No. 7 was carried out. The initial half of the first protein peak emerging from the column was dialysed and tested for antibody. This fraction gave positive staining of larvae with anti-μ chain reagent and less but detectable staining with anti-γ chain reagent (Fig. 13).

In control sections, no staining of larvae was observed with anti-heavy chain reagents alone or with these reagents after treatment of sections with sera or intestinal extracts from uninfected animals. As noted previously, the immunofluorescent reagents stained certain lymphoid cells surrounding cysts and anti-γ reagent gave some diffuse staining between muscle fibres and occasionally in areas surrounding the cysts. The yellowish fluorescence of certain granulocytes was prominent in areas of some sections.

DISCUSSION

The assumption has been made in this study that most cells stained for a particular heavy chain (α, γ or μ) contained all the component parts of IgA, IgG or IgM. This assumption was based on the evidence and arguments in Bernier and Cebra (1965) and Cebra and Bernier (1966). Also it had been observed that anti-light chain reagents stained the numerous α-chain containing cells in the intestinal mucosa thus indicating the presence of both component polypeptide chains of IgA.

The distribution of lymphoid cells containing different immunoglobulin classes in the intestinal mucosa of the rabbit agrees, in general, with the findings based on alternate single staining reported for the human intestine (Crabbé, Carbonara and Heremans, 1965;

Rubin, Fauci, Sleisenger and Jefferies, 1965). The presence of immunoglobulins in human intestinal secretions, with a relatively high percentage IgA in comparison with serum, has been noted (Chodirker and Tomasi, 1963). Similarly the results of Ouchterlony analyses suggest a relatively high ratio of IgA to IgG in gut extracts and intestinal contents of the rabbit, although no quantitative measurements of immunoglobulin were made in the present study. The failure to detect μ heavy chain in rabbit intestinal contents or wall extracts may be correlated with the relatively small number of these cells present in the mucosa, and only trace amounts of IgM have been reported in a variety of human non-vascular fluids, with the exception of colostrum (Chodirker and Tomasi, 1963). Then, too, IgM may be more susceptible to degradation by the enzymes in the intestinal homogenate than IgA or IgG.

The most striking change in relative proportions of immunoglobulin-containing cells in the mucosa of *Trichinella* infected animals was an increase in cells containing μ chain in early infection. No absolute changes in numbers of immunoglobulin-containing cells were measured, but the observations indicate an early infiltration or differentiation of IgM cells in greater proportions than IgG cells. Later in infection, and particularly after hyperinfection, IgG cells apparently made up a relatively larger proportion of the total IgM plus IgG cell population. Interestingly, this change in relative proportions of IgM and IgG cells with time after infection correlates roughly with the sequence of appearance of the immunoglobulin classes of humoral antibody as they can be detected after antigenic stimulation (Bauer, Mathies and Stavitsky, 1963).

Cells containing IgA were in high concentration in all the intestines examined, and it was considered that moderate changes in relative proportions of these cells would not be detected consistently for technical reasons: IgA cells were obviously in higher relative proportions in the villi than in the lower lamina propria and the plane of tissue sectioning could bias the result; IgA cells in the villi were often compact and without well delineated borders and their numbers could only be estimated from the visible nuclei. Finally, there was a tendency in counting to look for cells stained with the contrasting fluorochrome among the large numbers of cells stained for IgA. Consequently, a probable underestimation of the IgA numbers occurred.

A cellular infiltration of the intestinal mucosa has been reported as a prominent part of the host response to *Trichinella* infection (Larsh and Race, 1954). The present study indicates that immunoglobulin-containing cells participate in this response and exhibit changes in the relative distribution of immunoglobulins. However, the actual significance of these lymphoid cells as a local source of antibody was not critically investigated. Antibody to *Trichinella* larvae (IgG) was found in intestinal extracts, but at a time when antibody was also present in the serum and undoubtedly diffused throughout the vascular mucosa. No IgA antibody was detected, although this failure may be due to the inadequacies of the detection method employed.

The relatively low concentration of IgA-containing cells in the spleen and lymph nodes has been noted in another study of rabbit lymphoid tissues and can be correlated with the low serum level of this immunoglobulin (Cebra *et al.*, 1966).

The timing of the relative increase in IgM-containing cells in the spleen and popliteal lymph nodes in infection with *Trichinella* suggests a possible correlation with host response and antibody production to larvae. The relatively high ratio of IgM to IgG cells was observed in late infection and after hyperinfection at a time when titres to larval antigens are increasing. In infected rabbits antibody may be detected soon after infection; however,

significant antibody to larvae is usually found between the 3rd and 4th week of infection (Sadun, Anderson and Williams, 1962; Oliver-González, 1941). The immunological significance of the relative increase in IgM cells is uncertain. If a relationship exists between the proportion of cells containing a specific immunoglobulin and the relative serum concentration of their product (Cebra *et al.*, 1966), the absolute concentration of IgM in the sera of *Trichinella* infected animals might be expected to be increased in infection, a finding which has not been reported to our knowledge. However, at least one condition reported to give a sustained macroglobulin response, continued antigenic stimulation (Uhr and Finkelstein, 1963; Torrigiani and Roitt, 1965), may be present in *Trichinella* infections due to the larvae which are retained indefinitely. Although humoral IgM antibody reactive with larvae was detected in this study, no quantitative measurement of its concentration was made.

The relative proportions of IgM and IgG cells in the spleen of antigen-injected animals, as well as the untreated animals (Nos. 13 and 14) which had large numbers of immunoglobulin-containing cells in the spleen, indicated a shift in cell population toward IgM-containing cells when a comparison was made with the IgM–IgG cell proportions in the spleens of uninfected animals 11 and 12 and with the animals in early *Trichinella* infection. However, any general relationship of a high proportion of IgM cells in lymphoid tissues to hyperimmunization has not been confirmed by previous studies of rabbits immunized with proteins, hapten–protein conjugates, or *Salmonella* (Cebra *et al.*, 1966; Cebra and Bernier, 1966). Additional investigation is needed to elucidate the conditions which induce a high relative proportion of IgM cells in lymphoid tissues.

The presence of plasma cells, occasionally in large numbers, has been noted by a number of investigators in *Trichinella* infected muscles (Gould, 1945). In the present study, the number of immunoglobulin-containing cells in the diaphragms suggests that skeletal muscle is an important site for immunoglobulin production in *Trichnella* infections.

The cellular distribution of immunoglobulin classes in the diaphragm generally paralled that in the spleen and lymph nodes of the infected rabbits. The relative proportion of cells containing IgA observed in the diaphragm was somewhat higher than that found in the spleen, but a more extensive comparison would be required to evaluate the significance of this difference.

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REFERENCES

Bauer, D. C., Mathies, M. J. and Stavitsky, A. B. (1963). 'Sequences of synthesis of γ-1 macroglobulin and γ-2 globulin antibodies during primary and secondary responses to proteins, salmonella antigens and phage.' *J. exp. Med.*, **117**, 889.

Bernier, G. M. and Cebra, J. J. (1965). 'Frequency distribution of α, γ, κ and λ polypeptide chains in human lymphoid tissues.' *J. Immunol.*, **95**, 246.

Cebra, J. J. and Bernier, G. M. (1966). 'Quantitative relationships among lymphoid cells differentiated with respect to class of heavy chain, type of light chain and allotypic markers.' *Symposium on the Ontogeny of the Immune Response.* University of Florida Press (In press).

Cebra, J. J., Colberg, J. E. and Dray, S. (1966). 'Rabbit lymphoid cells differentiated with respect to α,-γ,-and μ-heavy polypeptide chains and to allotypic markers Aa1 and Aa2.' *J. exp. Med.*, **123**, 547.

CEBRA, J. J. and GOLDSTEIN, G. (1965). 'Chromatographic purification of tetramethylrhodamine-immune globulin conjugates and their use in the cellular localization of rabbit γ-globulin polypeptide chains.' *J. Immunol.*, **95**, 230.

CEBRA, J. J. and ROBBINS, J. (1966). 'Isolation and characterization of γA-immunoglobulin from rabbit colostrum.' *J. Immunol.*, **97**, 12.

CHODIRKER, W. B. and TOMASI, T. B., Jr. (1963). 'Gamma-globulins: quantitative relationships in human serum and nonvascular fluids.' *Science*, **142**, 1080.

COHEN, S. and PORTER, R. R. (1964). 'Structure and biologic activity of immunoglobulins.' *Advanc. Immunol.*, **4**, 287.

CRABBÉ, P. A., CARBONARA, A. O. and HEREMANS, J. E. (1965). 'The normal human intestinal mucosa as a major source of plasma cells containing γA-immunoglobulin.' *Lab. Invest.*, **14**, 235.

CRANDALL, R. B. (1965). 'Chemotactic response of polymorphonuclear leukocytes to *Trichinella spiralis* and *Ascaris suum* extracts.' *J. Parasitol.*, **51**, 397.

GOULD, S. E. (1945). *Trichinosis*. Thomas, Springfield.

KAGAN, I. G. (1960). 'Trichinosis: a review of biologic, serologic and immunologic aspects.' *J. infect. Dis.*, **107**, 65.

LARSH, J. E., Jr. (1963). 'Experimental trichinosis.' *Advanc. Parasitol.*, **1**, 213.

LARSH, J. E., Jr. and RACE, G. J. (1954). 'A histopathologic study of the anterior small intestine of immunized and non-immunized mice infected with *Trichinella spiralis*.' *J. infect. Dis.*, **94**, 262.

MCKELVEY, E. M. and FAHEY, J. L. (1965). 'Immunoglobulin changes in disease: Quantitation on the basis of heavy polypeptide chains, IgG (γG), IgA (γA), and IgM (γM), and of light polypeptide chains, type K (1) and type L (11).' *J. clin. Invest.*, **44**, 1778.

MELLORS, R. C. and KORNGOLD, L. (1963). 'The cellular origin of human immunoglobulins (γ_2, γ1M, γ1A).' *J. exp. Med.*, **118**, 387.

OLIVER-GONZÁLEZ, J. (1941). 'The dual antibody basis of acquired immunity in trichinosis.' *J. infect. Dis.*, **69**, 254.

RUBIN, W., FAUCI, A. S., SLEISENGER, M. H. and JEFFERIES, G. H. (1965). 'Immunofluorescent studies in adult celiac disease.' *J. clin. Invest.*, **44**, 475.

SADUN, E. H., ANDERSON, R. I. and WILLIAMS, J. S. (1962). 'Fluorescent antibody test for the serological diagnosis of trichinosis.' *Exp. Parasit.*, **12**, 423.

TORRIGIANI, G. and ROITT, I. M. (1965). 'The enhancement of 19S antibody production by particulate antigen.' *J. exp. Med.*, **122**, 181.

UHR, J. W. and FINKELSTEIN, M. S. (1963). 'Antibody formation, IV. Formation of rapidly and slowly sedimenting antibodies and immunological memory to bacteriophage Φ X174.' *J. exp. Med.*, **117**, 457.