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EXPERIMENTAL AUTOIMMUNE GASTRITIS IN THE RHESUS MONKEY

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SUMMARY

Three rhesus monkeys were injected with pooled monkey gastric mucosa suspension plus complete Freund adjuvant. All three animals developed organ-specific autoantibodies and delayed skin tests to gastric antigen. The circulating antibodies were demonstrated by tanned cell haemagglutination using a well-centrifuged, heated gastric extract, and by complement fixation tests employing a lightly centrifuged gastric suspension. In immunofluorescent tests, antibodies localized primarily in the parietal cell cytoplasm of the gastric mucosa. All three monkeys showed histological evidence of chronic multifocal gastritis consisting of monocytic infiltration of the mucosal layer with atrophy of the neighbouring glands. The thyroids and other organs of these monkeys were normal. Normal monkeys, or control monkeys injected with monkey thyroid, adrenal or testis extract did not show these gastric changes.

INTRODUCTION

Attempts to produce experimental autoimmune gastritis in animals were made by several investigators. Taylor in 1959 was unable to find gastric lesions in rats immunized with homologous gastric mucosa; similar negative results were obtained in the guinea-pig (Coghill, 1960). On the other hand, Hennes *et al.* (1960) and Fixa *et al.* (1964) injected dogs with human or canine gastric juice or gastric mucosa extract incorporated in complete Freund adjuvant and obtained atrophic gastritis.

In viewing human autoimmune disease, a parallel has been drawn between thyroiditis and pernicious anaemia (Irvine *et al.*, 1962, Markson & Moore, 1962a, b; Doniach, Roitt & Taylor, 1963). Studies of the different antigens involved in the autoimmune response of pernicious anaemia showed that there are antibodies to soluble antigen (intrinsic factor) and particulate antigen (partietal cells) in the serum of most of the patients suffering from

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this disorder (Taylor *et al.*, 1962), analogous to the autoantibodies to soluble thyroglobulin and to cytoplasmic particulates found in most patients with chronic thyroiditis.

Recent investigations have revealed that the rhesus monkey is a particularly suitable animal for the development of experimental autoimmune thyroiditis (Rose *et al.*, 1965; Kite *et al.*, 1965; Kite, Argue & Rose, 1966; Doebbler & Rose, 1966; Rose *et al.*, 1966; Andrada, Rose and Kite, 1968). Like other experimental animals, it produces precipitating and agglutinating antibodies to thyroglobulin following injection of homologous or related thyroid extracts. In addition, it develops complement-fixing and cytotoxic antibody to antigens of the thyroid epithelial cells. Because the monkey seems capable of producing autoantibodies to both soluble and cellular antigens it was selected for investigations on the experimental induction of autoantibodies to gastric mucosa and of lesions in the target organ, the stomach.

MATERIALS AND METHODS

Three rhesus monkeys (*Macaca mulatta*), approximately 2–3 years of age and weighing 4–5 lb, were immunized with gastric mucosa crude extract as described below. Monkeys 'Red' and 'Wen' were female and monkey 'Sid' was a male. As a control measure, sera and tissue from four monkeys immunized in an analogous fashion with thyroid extract, four monkeys immunized with testis extract, and two immunized with adrenal extract were included in this study.

Preparation of antigens

A crude suspension of monkey gastric mucosa was prepared from fresh stomach as follows: the stomach was opened immediately after death, cleaned with tapwater and then cold phosphate-buffered saline (pH 7·2), and blotted with tissue paper to remove most of the mucus. Then it was frozen in a Petri dish with the mucosal surface upward. When it was well frozen the fundal portion was scraped with a sharp scalpel. The specimen thus obtained was weighed, minced with scissors and mixed with a double amount (weight per volume) of buffered saline at pH 7·2. It was then disrupted in a Teflon type blender for 5 min in the cold, stirred overnight in the cold room and centrifuged at 800 *g* in a refrigerated centrifuge for 5 min; the supernatant was used as antigen. It was mixed with an equal volume of complete Freund's adjuvant (Difco) fortified with 3·5 mg of killed *Mycobacterium tuberculosis* H 37Ra. This mixture was thoroughly emulsified and then injected intradermally between the digits of each foot and hand of the monkeys. The injections were repeated at approximately monthly intervals as shown in the tables.

To perform haemagglutination tests, the crude suspension of gastric mucosa was further centrifuged at 69,000 *g* for 30 min and protein concentration determined by biuret analysis. For some tanned cell haemagglutination tests this clarified extract was boiled for 2 min.

In some experiments, the scrappings were treated with 0·1% ficin solution in buffer. After digestion with enzyme for 1 hr at 4°C, the suspension was centrifugated in the cold (6000 *g* for 20 min).

Control extracts

Other organs from the monkey and other laboratory animals (guinea-pig, rat, rabbit and mouse) were obtained immediately after death. The crude suspensions or extracts were prepared in a similar way.

Antigen fractionation

Microsomal, mitochondrial and nuclear fractions from the stomach as well as from liver extract were prepared following Hogeboom's method (Hogeboom & Schneider, 1955). Protein concentration in all fractions was checked by the biuret method.

Thyroid hormone determinations

Serum samples from monkeys 'Sid' and 'Red' were analysed for the presence of thyroid hormones in the sera at different stages of immunization (Bio-Science Laboratories, Van Nuys, California). The normal values for protein bound iodine (PBI) in twelve normal monkeys were found to be 2·5–4·5 mg/100 ml and butanol-extractable iodine (BEI) 2·5–3·5 mg/ml.

Haematological tests

Complete blood counts, including values for haemoglobin, haematocrit, sedimentation rate and white cells was performed in monkeys 'Red' and 'Sid' according to usual laboratory procedures; also bone marrow examination was done at the time of the autopsy of both animals.

Serological tests

Tanned cell haemagglutination: The method described by Witebsky & Rose (1956), as well as a micro-method adapted for this investigation, was used. The micro-method was performed by adding the following to plastic plates: 0·025 ml antiserum dilution and 0·025 ml of tanned and coated human (Group O) erythrocytes. Plates were then shaken and readings were taken after 1 and 24 hr at 4°C. The pattern of sedimentation was similar to the one obtained with the standard method.

Complement fixation

The micro-technique of Blizzard *et al.* (1962) was performed in plastic plates using 0·025 ml of antiserum dilution (previously inactivated at 56°C for 30 min), 0·025 ml of tissue suspension diluted 1:10 in modified barbital buffer, and 0·050 ml of guinea-pig complement diluted 1:20. Plates were incubated for 2 hr at 37°C and then sensitized sheep cells (0·05 ml) were added. After a further incubation for 30 min the plastic plates were centrifuged for 5 min at 1500 rev/min in the International Refrigerated centrifuge. The extent of haemolysis was determined by the size of the erythrocyte pellet and the colour of the supernatant. Results are graded from ++++ (no haemolysis) to negative (complete haemolysis); positive and negative controls were included in each test.

Indirect immunofluorescence

Monkey and rat stomachs were used as substrate for the indirect immunofluorescent studies. Fresh blocks from the whole thickness of the wall of the fundus of the stomach were obtained after killing the animal; they were snap frozen on solid CO_2 and used

immediately or within 2 weeks of storage in the freezer. Sections (6 μ) were cut in a cryostat at $-20°C$, mounted on cleaned glass slides without any slide adhesive, dried with air. In some experiments slides were treated with cold acetone for 2 min to fix the sections.

Experimental monkey and control sera, which were tested at different dilutions, were incubated on the sections for 30 min at room temperature in a damp atmosphere. After rinsing and washing for about 45–60 min in buffered saline, pH 7·2, the slides were treated with anti-human γ-globulin rabbit serum conjugated with fluorescein isothiocyanate. After another washing of 45 min slides were mounted in glycerol and observed under an American Optical Company fluorescent microscope.

Complement levels

The method of Kabat (1961) was employed, and the levels of complement expressed as $C'H_{50}$ units.

Double diffusion gel precipitation (Ouchterlony)

Agar or agarose at concentrations from 0·8 to 1·2 g/100 ml in phosphate buffer, pH 7·0 to 7·2 (0·15 M) were used as the gelified medium; wells of various diameters were cut at different distances from each other based on trial-and-error. After filling the wells with antisera and clarified extracts, the plates were kept in a moist chamber at room temperature. Readings were taken at 24, 48 and 72 hr.

Skin tests

A well centrifuged and membrane-filtered gastric mucosa extract with a protein concentration of 1·5% was injected intradermally in 0·1 ml volume in the shaved abdomen of the monkeys. As controls the same amount of buffered saline and a 1% extract of killed tubercle bacilli were also injected in the opposite side. Readings were taken at 24 and 48 hr.

Histopathology

Gastric biopsies were taken at different intervals. Monkeys were prepared by fasting for 12 hr; anaesthesia (sodium pentobarbital) was given intravenously; abdomen was opened in the supraumbilical mid-line. After presentation of stomach, its fundus was clamped with Pean gastro-intestinal forceps. Visceral peritoneum as well as the other layers of the stomach were opened and a small piece (about 8×8 mm) of the gastric wall was removed. The organ was then sutured in three different layers with a special silk for vascular surgery. Sulphathiazole powder was introduced in the peritoneal cavity before closure.

Organs were fixed in 10% formaldehyde. Histological sections were made after paraffin embedding and stained by the haematoxylin and eosin, periodic acid–Schiff or Pappenheim stain methods. Thyroid biopsies were described in another publication (Andrada *et al.*, 1968).

RESULTS

Immunization

Monkey 'Red' (Table 1) was followed for a period of 92 days during which it received three injections of monkey gastric mucosa suspension with complete Freund's adjuvant

augmented with *M. tuberculosis*. A week after the first injection it developed a moderate titre in the tanned cell haemagglutination test which persisted until the 56th day. The antibody again appeared in moderate titre near the end of the observation period. By means of complement fixation tests antibodies were shown from the 15th to the 92nd day in low to moderate titres. When thyroid extract was used as antigen in either test, results were always negative. Complement levels decreased progressively to a value of 14 units near the end of the experiment. Histologically the stomach and the thyroid were normal on the 56th day. The stomach at autopsy showed marked infiltration (+ +) predominantly in the mucosa and submucosa (Fig. 1). Many fundic glands lost their normal appearance and the border of the mucosa was sometimes disrupted. Fig. 2 shows in greater magnification the infiltrating elements within the glands and especially in areas surrounding the parietal cells. Because of the patchy distribution and severity of the infiltration this type of gastritis could be

TABLE 1. Experimental gastritis in monkey 'Red' (female)

Day	HA	CF	C'50 units	IF	Histopathology
0	0	0	46	0	
0	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
8	32	0	50	0	
15	128	2	34	0	
22	64	8	28	0	
29	512	8	34	5	
36	64	32	39	0	
36	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
50	32	4	26	—	
56	0	4	19	10	
60	Biopsies of stomach and thyroid				Negative
70	0	4	25	0	
78	0	4	—	—	
78	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
87	16	16	14	—	
92	32	16		10	
92	Autopsy				+ +

Autopsy findings: no lesions in thyroid, adrenal, parotid, lung, spleen, kidney, sciatic nerve or bone marrow. Gastric lesions were graded on a scale of negative to + + + (see Figs. 1–7).

HA, tanned cell haemagglutination; CF, complement fixation; C'50 units, serum complement levels; IF, indirect immunofluorescence.

considered as chronic multifocal. Autopsy performed at the 92nd day showed no abnormalities in thyroid, adrenal, lung, parotid, spleen, kidney, sciatic nerve and bone marrow.

The histological examination of many normal monkey stomachs showed, besides the lymphatic follicles common in all gastro-intestinal tracts, some with few infiltrating lymphoid cells in the tunica propria; the follicles were usually surrounded by a fibrotic 'membrane-like' structure, as seen in the transverse section of a normal monkey stomach (Fig. 3). In Fig. 4 a lymph node is seen between the tunica propria and submucosa in a normal mucosa.

Monkey 'Sid' was observed for a period of 525 days after the first injection (Table 2). This animal received fourteen injections of gastric mucosa suspension plus complete

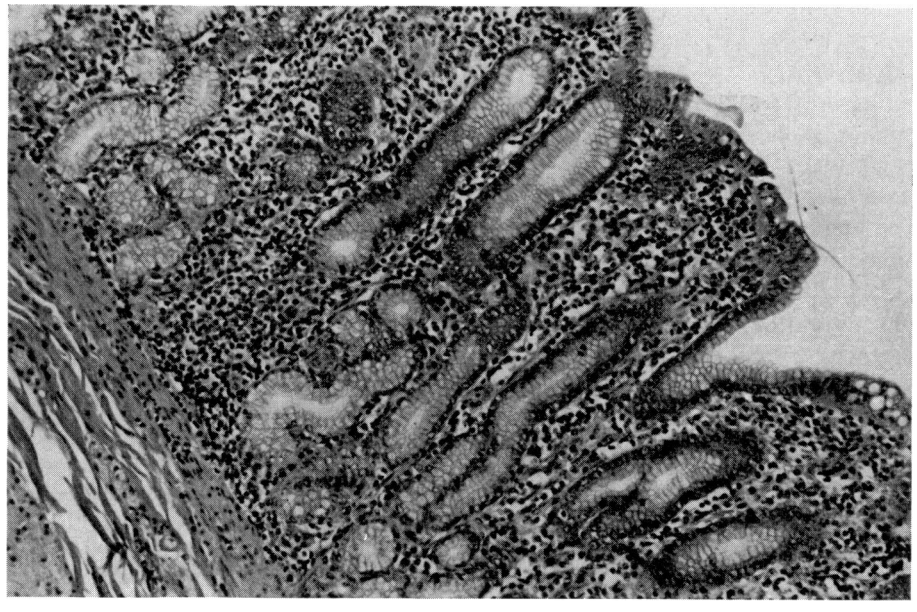

FIG. 1. Histological appearance of gastric mucosa of monkey 'Red', killed after 92 days of immunization. Note disruption of the architecture of the mucosa with infiltration of lymphocytes in submucosa and between the fundic glands reaching the apical border. There is a relative decrease of parietal cells. H & E, ×40.

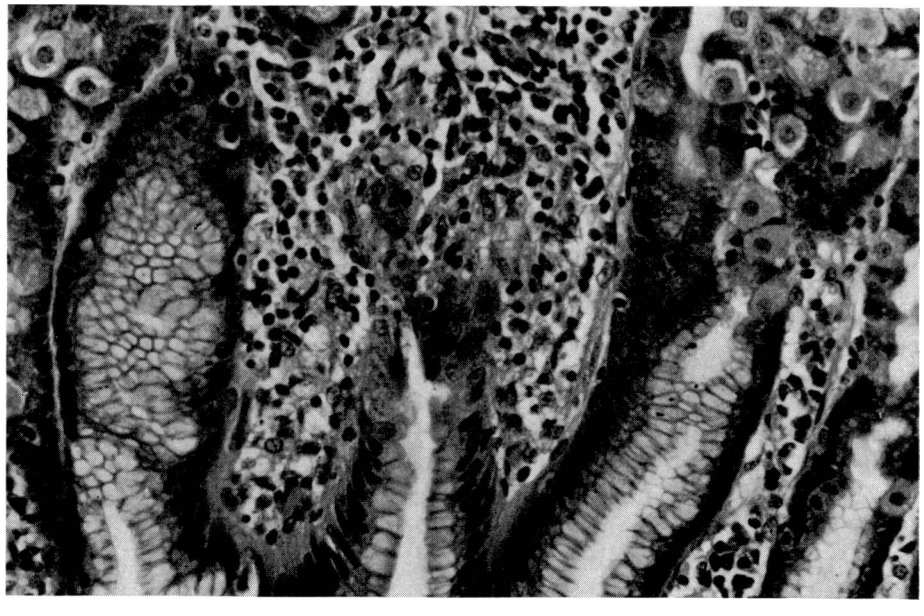

FIG. 2. Same section as Fig. 1 at higher magnification. The infiltrating elements within the glands and in the connective tissue which surrounds these structure are shown. Small and large lymphocytes and plasma cells can be identified in the infiltrate. H & E, ×160.

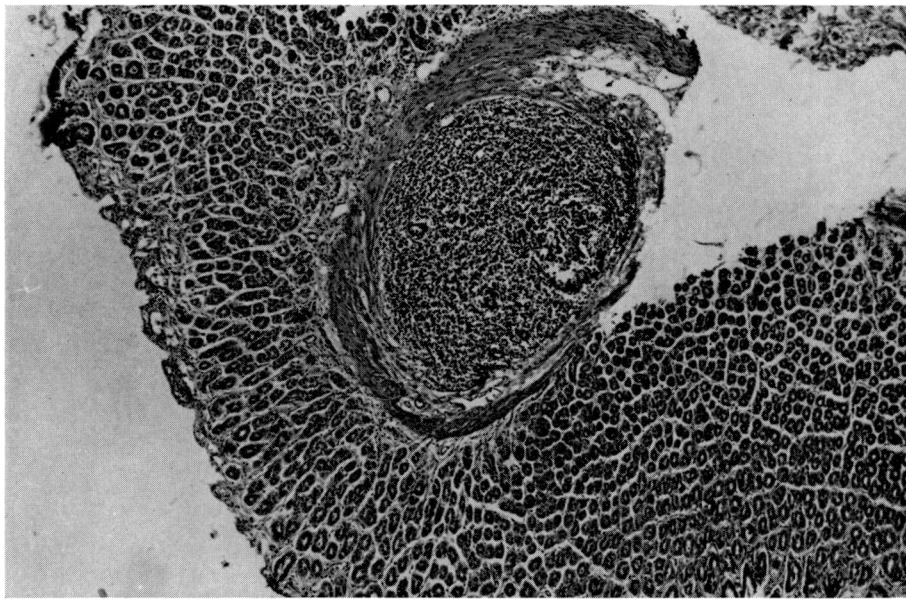

FIG. 3. Histological appearance of the gastric mucosa of a normal monkey. Cross-section of a lymph node in the muscularis and submucosa is seen, surrounded by a fibrotic capsule. H & E, ×40.

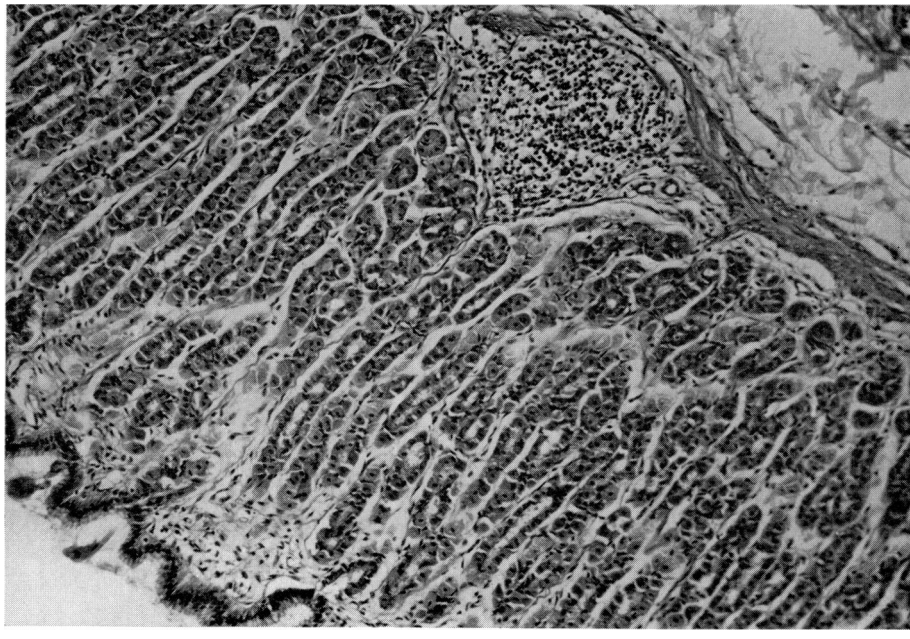

FIG. 4. Histological appearance of the gastric mucosa of a normal monkey. Note the regular linear arrangement of the glands with little connective tissue. There is a lymph node between the tunica propria and submucosa. H & E, ×50.

TABLE 2. Experimental gastritis in monkey 'Sid' (male)

Day	HA	CF	C'50 units	IF	Histopathology
0	0	0	40	0	
0	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
8	0	0	45	0	
15	64	4	32	0	
22	256	8	30		
29	512	32	27	0	
36	128	4	58		
38	Biopsies of stomach and thyroid				Negative
49	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
50	512	4	45	5	
56	512	16	39		
64	8	16			
70	256	8	32		
78	256	4			
87	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
87	256	8	20	10	
98	512		41	10	
108	Biopsy of thyroid				Negative
108	256	8	30		
133	128	4	27		
139	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
175	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
199	64	64			
206	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
221	Biopsy of stomach				++
221	1024	128	27		
252	256	128	30		
252	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
269	256	32			
311	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
326	1024	128	25		
339	1024		42		
343	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
343	512	128	28	10	
357	Biopsy of stomach				+++
357	2048	128	37		
371	2048		31		
373	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
374	Skin test negative				
416	1024	32	40		
416	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
435	1024	32			
449	64	16	23		
449	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
462	Biopsy of stomach				+++
462	32	16	20		
487	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant				
505	Skin test negative				
505	32	16			
514					
525	32	16			
525	Autopsy				+++

Autopsy findings: see text.

Freund's adjuvant. Two thyroid and four gastric biopsies were performed during this period. After the 2nd week tanned cell haemagglutinating and complement fixing antibodies appeared in moderate titres; they reached a maximum in about 1 year (357–371 days) after which titres were lower. Complement levels showed variations, reaching low values of 20 units at the 87th day and at the 462nd day. This last determination represents the value at the time of a gastric biopsy, which revealed evidence of inflammation. No anti-thyroid antibodies could be detected in any of the sera from this monkey. Skin tests with gastric antigen applied at 374 and 505 days were negative.

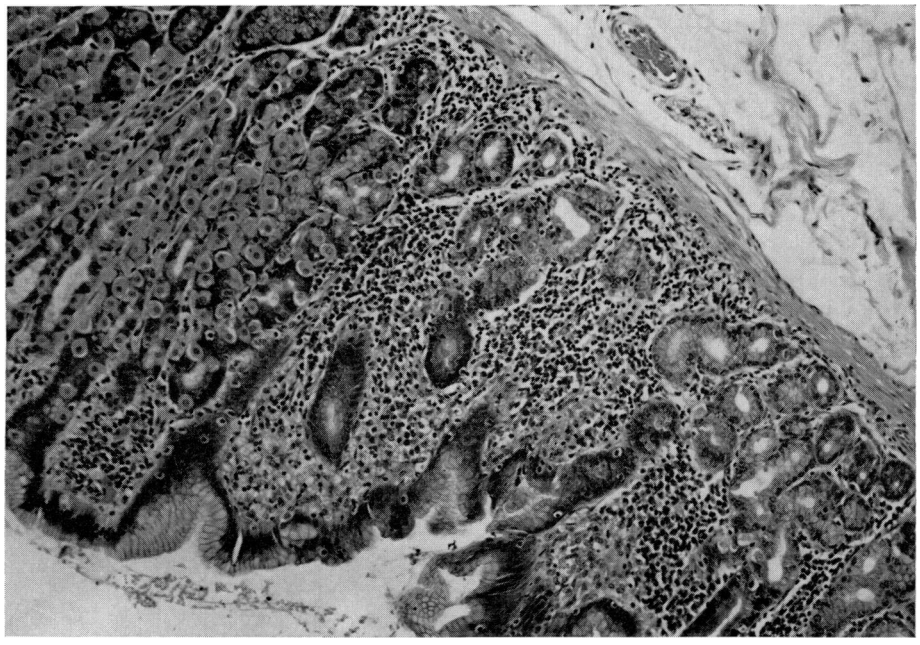

FIG. 5. Histological appearance of the gastric mucosa of monkey 'Sid', biopsied at 357 days after immunization. Some parts of the mucosa (left portion of picture) exhibit an almost normal appearance; the rest shows interstitial infiltrate invading numerous oxyphilic glands. There are some scattered neutrophils, some plasma cells but the majority of elements are lymphocytes. There is hyperaemia in capillaries and oedema in tunica propria. H & E, × 50.

Thyroid biopsies taken at 36 and 108 days and sections at the time of killing were normal in appearance. A gastric biopsy taken at 36 days was normal; at 221 days it showed moderate infiltration, giving a histological picture similar to monkey 'Red'. At 357 and 462 days biopsies showed hyperaemia, oedema in tunica propria and a diffuse infiltration in many areas (Fig. 5). Some glands were invaded by mononuclear cells; the submucosa and some times the muscularis levels were also involved. Areas where there was a predominance of oxyphilic glands showed infiltration between the glands, in the apical and basal (lower third) portion. Some glands appeared necrotic (Fig. 6); with higher magnification the spaces between glands could be observed to be filled with various cellular elements, mostly lymphocytes and numerous plasma cells (Fig. 7) (better identified with Pappenheim strain), all located in a spongy mucosa. This appearance was produced by the vacuolization of

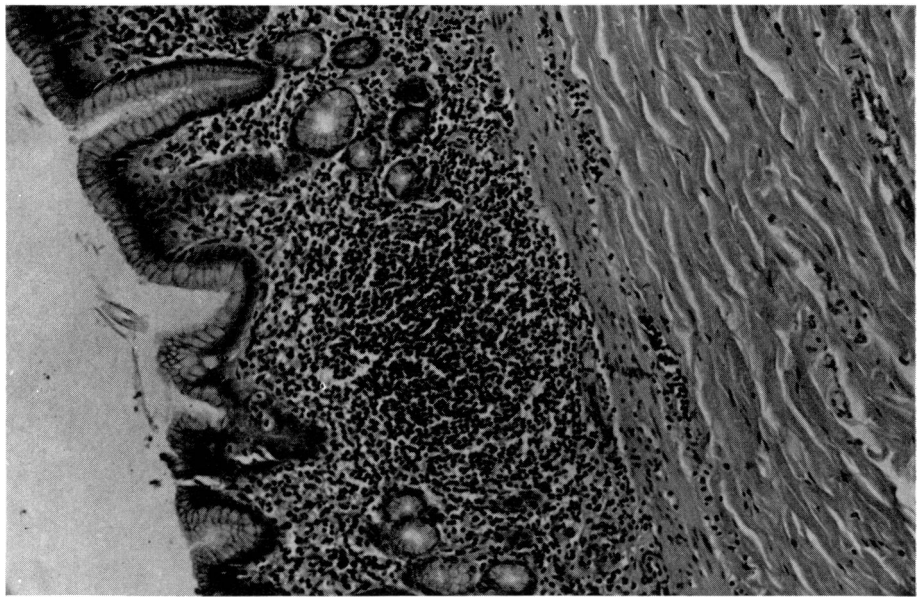

FIG. 6. Histological appearance of the gastric mucosa of monkey 'Sid' 525 days after immunization. Intense infiltration resembles a germinal centre in mucosa. Interstitial oedema and infiltration are seen in submucosa. H & E, × 50.

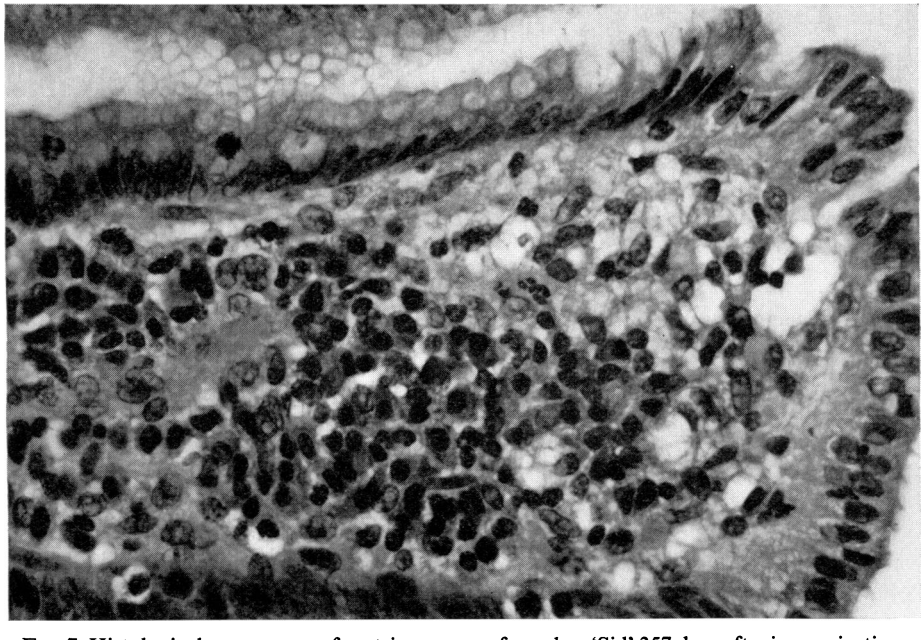

FIG. 7. Histological appearance of gastric mucosa of monkey 'Sid' 357 days after immunization. Higher magnification of the interglandular spaces showing types of infiltrating elements: lymphocytes, plasma cells and few neutrophils. The mucosa has a foamy aspect produced by the vacuolization of many cells and oedema of the interstitial tissue. H & E, × 160.

many cells and the oedema of the intercellular connective tissue. At 525 days this monkey was killed. A complete autopsy showed in the liver few collections of infiltrating cells near the portal spaces; some hyperaemia was evident in the adrenal medulla. The rest of the organs examined were normal.

Haematological studies in these two monkeys showed decreased values for haematocrit and haemoglobin with normal sedimentation rate and a moderate leucocytosis. The anaemia was normocytic, normochromic type according to the haematological values. The bone marrow was normal. Biochemical studies demonstrated no abnormality in thyroid hormone production, as measured by PBE and BEI.

TABLE 3. Experimental gastritis in monkey 'Wen' (male)

Day	HA	CF	Histopathology
0	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant		
0	Biopsy of stomach		Negative
21	256	8	
21	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant		
32	128	4	
46	512	4	
60	256	8	
60	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant		
82	256	8	
82	Biopsy of stomach		++
85	Injected with 1·0 ml monkey gastric mucosa suspension plus complete Freund's adjuvant		
85	128	4	
111	1024	128	
123	32	16	
123	Autopsy		++

Autopsy findings: see text.

Monkey 'Wen' received four injections of the same antigenic preparation and was followed for 123 days (Table 3). At the 3rd week it showed moderate titres of tanned cell haemagglutinating antibodies and low titres of complement-fixing antibodies. The highest values were reached at 111th day, with the tanned cell haemagglutination titre of 1024 and the complement fixation titre of 128. Complement levels were not determined in this animal; anti-thyroid antibodies were not present.

Two gastric biopsies were obtained; the first at 21 days, was normal and the second at 82 days was moderately positive (++). The histological picture was very similar to that previously described for monkeys 'Red' and 'Sid'. At the autopsy no infiltration was found in ileum, testis, thyroid, kidney and liver.

Organ specificity

Using crude suspensions of different organs, adjusted to the same protein concentration, a test was performed by complement fixation with serum of the monkeys 'Red' and 'Sid', and of a normal monkey (Table 4). After immunization both monkeys reacted with high

TABLE 4. Complement fixation test: reaction with stomach and other gastro-intestinal suspensions of sera from monkeys immunized with gastric mucosa and from normal monkey

Organ suspension*	Dilution of antigen	Monkey 'Red'		Monkey 'Sid'		Normal monkey
		Pre-injection	Post-injection	Pre-injection	Post-injection	
Stomach	Undiluted	+++	++++	+++	++++	++++
	1:3	+	++++	+	++++	−
	1:9	−	++++	−	++++	−
	1:27	−	++++	−	++++	−
	1:81	−	−	−	−	−
Colon	Undiluted	++++	++++	++++	++++	++++
	1:3	++	+	−	+	−
	1:9	+	−	−	−	−
	1:27	−	−	−	−	−
Ileum	Undiluted	+++	++++	++	+++	+++
	1:3	+	+	+	+	+
	1:9	−	−	−	−	+
Oesophagus	Undiluted	++++	+++	++	++	++
	1:3	+	+	−	−	−
	1:9	−	−	−	−	−
Parotid	Undiluted	−	−	−	−	−
	1:3	−	−	−	−	−
	1:9	−	−	−	−	−

++++, No haemolysis; +++, slight haemolysis; ++, moderate haemolysis; +, almost complete haemolysis; −, complete haemolysis.

* Protein concentration of all extracts, 1·5–1·8 mg/100 ml. Antiserum dilution, 1:32.

TABLE 5. Tanned cell haemagglutination test: reaction with stomach, colon, ileum and oesophagus mucosal extracts of monkeys immunized with gastric mucosa extracts

Organ extract	Haemagglutination titres				
	Monkey 'Red'		Monkey 'Sid'		Normal monkey*
	Pre-injection	Post-injection	Pre-injection	Post-injection	
Stomach	—	64	—	512	—
Colon	—	2	—	—	—
Ileum	—	—	—	—	—
Oesophagus	—	—	—	—	—

* Using these antigens, ten other normal monkey sera were examined. Nine were negative and one positive with gastric extract in a dilution of 1:4.

dilutions of gastric suspension and not at all with low dilution of colon, ileum, oesophagus and parotid gland suspensions. Pre-injection serum samples of both animals, as well as of the normal monkey, reacted with the lower dilutions of gastro-intestinal suspensions. Using human red cells coated with the clarified extracts of the same organs, the

TABLE 6. Tanned cell haemagglutination and complement fixation tests: serum of monkey 'Sid' tested with pooled monkey stomach extract and its own gastric extract

Days following injection	Titre by tanned cell haemagglutination,* extract of:		Titre by complement fixation,† extract of:	
	Pooled gastric mucosa	Monkey 'Sid' gastric mucosa	Pooled gastric mucosa	Monkey 'Sid' gastric mucosa
326	128	16	128	16
371	16	16	8	4
435	64	32	8	4
449	32	8	8	4
462	32	16	32	8
505	32	32	8	8
525	32	32	16	8

* Antigen diluted 1:50, centrifugated 69,000 *g* for 30 min and boiled 2 min.

† Antigen diluted 1:10, centrifugated 800 *g* for 5 min.

TABLE 7. Complement fixation test: serum of monkey 'Sid' tested with different monkey tissue fractions

Antigen*	Complement fixation titre
Gastric mucosa crude suspension	32
Gastric mucosa microsomes	32
Gastric mucosa mitochondria	16
Gastric mucosa crude suspension (ficin treated)	32
Liver crude suspension	0
Liver microsome fraction	0
Adrenal crude suspension	0
Muscle (smooth) crude suspension	0
Muscle (striated) crude suspension	0

* Protein concentration: 1·8–2·6 mg/ml.

monkey antisera reacted only with the cells coated with gastric mucosa (Table 5). Pre-injection samples and the normal serum were negative in this test.

In other experiments it was shown that the serum of monkey 'Sid' was able to react with its own gastric tissue, when the extract obtained from its stomach was used to coat the red cells in the tanned cell haemagglutination test and as antigen in the complement fixation test (Table 6).

Studies by complement fixation using different fractions of gastric mucosa and other organs revealed equivalent reactions with gastric mucosa, the crude extract, microsomes, and the crude extract treated with ficin; the mitochondrial preparation proved to be a slightly weaker antigen (Table 7). The other organs (liver, adrenal and muscle) or their microsomal fractions did not react.

TABLE 8. Complement fixation test: species specificity serum of monkey 'Sid'

Dilution of antigen	Gastric mucosa extracts*				
	Human	Monkey	Rabbit	Guinea-pig	Rat
Undiluted	++++	++++	+++	+++	++++
1:2	++++	++++	++	++	++++
1:4	++++	++++	++	+	+++
1:8	+++	+++	+	−	++
1:16	+++	+++	−	−	+
1:32	+	++	−	−	−
1:64	−	−	−	−	−
1:0	−	−	−	−	−

Antiserum dilution, 1:32.

* Supernatants of extracts ultracentrifugated at 800 *g* for 5 min. Protein concentration: 1·5 mg/100 ml.

TABLE 9. Indirect immunofluorescence: reactions of monkey antisera with monkey gastric mucosa and other organs

Immunized with:	Monkey	Immunofluorescence with sections of monkey:			
		Stomach	Thyroid	Testis	Other organs*
Gastric mucosa	'Red'	++	−	−	−
	'Sid'	++	−	−	−
Thyroid	'Unice'	−	++	−	−
	'Will'	−	++	−	−
Testis	'Tes-4'	−	−	++	−
	'Tes-5'	−	−	++	−

Antiserum dilution, 1:10. ++ indicates reaction.

* Liver, kidney, ovary and adrenal.

Species specificity

Table 8 shows the results obtained by complement fixation using decreasing amounts of supernatants of lightly centrifugated extracts of human, monkey, rabbit, guinea-pig and rat gastric mucosa. These extracts, in similar protein concentrations, were mixed with a

1:32 dilution of serum from monkey 'Sid'. There was cross-reaction with human and, in a less intensity, with rat gastric extracts. Very weak reactions were obtained with rabbit and guinea-pig extracts.

Immunofluorescence

Several sera of monkeys 'Red' and 'Sid' were analysed by indirect immunofluorescence; some of them reacted positively in dilutions up to 1:10, giving a pronounced fluorescence in the parietal cells of the gastric mucosa (Fig. 8). This reaction was not obtained with the pre-immune serum of the sera of monkeys immunized with adrenal, thyroid or testis (Table 9).

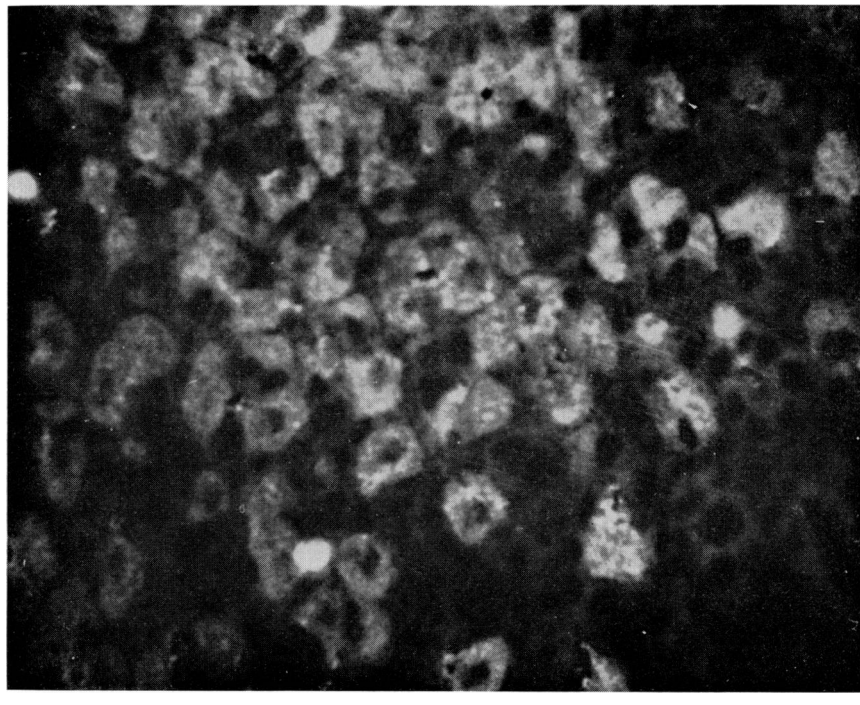

FIG. 8. Indirect immunofluorescence test of monkey serum 'Sid' showing localization in the parietal cells of the monkey gastric mucosa. × 160.

Using stomach sections of monkey 'Sid' this reaction was also obtained with antiserum of 'Sid', but only at a dilution of 1:4. Sera of monkeys 'Red' and 'Wen' were negative in indirect immunofluorescent tests using sections of 'Sid's' stomach.

DISCUSSION

The present investigations reveal that it is possible to induce specific autoantibodies to gastric mucosa by injection of monkeys with mucosa extract plus complete Freund's adjuvant. These antibodies were measured by two means, the tanned cell haemagglutination test and the complement fixation reaction. The antigen in tanned cell haemagglutination was a soluble saline extract of gastric mucosa; for this test it seems that with highly centri-

fugated material better results were obtained when the antigen was boiled for 2 min. This accords with previous observations on thyroglobulin (Witebsky & Rose, 1956). For the complement fixation test a lightly centrifuged mucosal suspension was generally utilized. The complement-fixing antigen could be assigned to the particulate portion of the gastric suspension, being found in the microsomal and mitochondrial fractions. The antigen responsible for tanned cell haemagglutination was in the soluble portion. It is still not clear whether the same antigen or two different antigens are involved in the two serological reactions.

Multiple injections of homologous gastric extract initiated gastritis as well as autoantibodies. Biopsies taken early in the course of immunization were normal in appearance. Later, after several injections were administered, lymphoid and plasmacytic infiltration of the mucosa and submucosa became apparent. It is not known whether these lesions would regress if repeated injections were not given. One could not correlate the appearance of these lesions with the titre of circulating autoantibodies. Skin tests of the monkeys traced the development of delayed hyperactivity to gastric extract. Based on a limited number of observations, no simple and direct correlation could be found. The lesions were confined to the stomach. Examination of other organs from the monkeys revealed no signs of injury with the exception of occasional scattered infiltrates in the liver of one of the animals ('Sid').

The complement fixing antigen involved in experimental gastritis seems to be quite similar to the cytoplasmic antigen of pernicious anaemia. It is found in the microsomal fraction and is resistant to ficin digestion (Baur, Roitt & Doniach, 1965). By immunofluorescence, the antigen can be localized in the gastric parietal cells with the distribution strikingly similar to that reported with pernicious anaemia sera.

The antibodies were specific for gastric antigen. No cross-reactions were observed with other monkey organ extracts and especially with other extracts of the intestinal tract. The presence of distinctive antigens of the stomach was first reported by Witebsky & Zeitzig (1932) using bovine material. A parallel study was carried out in this laboratory using human gastro-intestinal mucosa. Although many antigens were shared by all levels of the gastro-intestinal tract, a certain number of distinctive antigens were encountered. Rapp *et al.* (1964), identified nine organ specific antigens in the human stomach, two of which were shared with the small intestine.

The antibodies under discussion are to be considered autoantibodies because they reacted with extracts obtained from the gastric mucosa of the antibody-producing monkey as well as pooled gastric mucosa. Immunofluorescence experiments suggested a decrease in the reactivity of the gastric mucosa of the immunized monkey, possibly due to a reduction or blocking of the organ-specific antigen in the affected target tissue. A similar experience was encountered in experimental thyroiditis where the content in thyroglobulin of the thyroid gland was reduced corresponding to the degree of inflammation (Rose & Witebsky, 1956). In the case of experimental gastritis described here, that reduction never reached a stage of complete disappearance.

The gastric mucosa is known to be rich in blood group substance. For that reason, all monkey antisera were tested with human erythrocytes of Groups A, B and O. No elevated titres were found. Rabbits injected with Gram-negative bacteria were found to produce blood group specific antibodies even though the animals had these isoantigens in their own tissues (Saint Martin & Eyquem, 1967).

The antibodies also cross-reacted with gastric mucosa of certain other species. This finding provides additional evidence for the organ specific nature of the antigen. Saint Martin & Eyquem (1967) reported that heteroimmunization of rabbits with crude extracts of the stomach of monkey, dog, cat, guinea-pig and rat provoked the appearance of auto-antibodies demonstrable by complement fixation and immunofluorescence. Holborow, Asherson & Wigley (1963) reported that rabbits injected with homogenates of rat stomach, ileum and colon tissues produced autoantibodies to their own gut mucosa. No response was found to injections of rabbit intestinal antigens. They also showed several organ-specific antigens present in the mucous secretions and epithelial cells of the stomach, ileum and colon. There is no reference of any pathological changes in the respective organs.

In 1962 Hennes *et al.* reported that dogs injected with human or dog gastric juice in Freund's adjuvant developed atrophic gastritis marked by degenerative changes in the mucosa and leucocytic infiltration. Circulating antibodies and delayed skin reaction to gastric antigen were found. Some dogs eventually showed histamine-fast achlorhydria. They speculated that the human disease, atrophic gastritis, may be associated with autoimmunization to autologous gastric juice. Confirmatory results were reported by Fixa *et al.* (1964).

The biochemical and haematological data gathered on the autosensitized monkeys do not allow us to conclude that a process analogous to pernicious anaemia was initiated. Although haemoglobin levels generally fell in the course of immunization, there were no morphological changes in the red cells, the white blood cells or the bone marrows suggestive of pernicious anaemia. Unfortunately, it was impossible to perform vitamin B_{12} absorption studies or to seek antibody to intrinsic factor.

Of particular importance is the fact that the thyroid glands of the immunized animals were entirely normal in appearance. In addition, the antibodies produced failed to react with thyroid antigens. It seems clear, therefore, that the development of autoimmunity to gastric mucosa is entirely distinct from immunization to thyroid components. A clinical and epidemiological association of pernicious anaemia and thyroid autoimmunity has been reported by many investigators (Irvine, 1965). However, no evidence for an antigenic relationship between these two organs has been adduced. It seems likely, therefore, that the two processes of autosensitization in man are entirely separate. The fact that they are so frequently related may bespeak some more basic defect in immunological recognition. Why these two particular organs should be thus associated is still a matter of conjecture.

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