Pernicious anaemia autoantibody to gastric parietal cells

Immunofluorescence test with rat stomach

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SYNOPSIS Rat stomach provided an excellent substrate for immunofluorescence testing of gastric parietal cell autoantibodies in 65 human sera. The results of similar tests using human stomach corresponded closely in the 42 cases examined. The rat stomach had some advantage over human stomach in its ready availability in the fresh state, its occasional brighter staining reactions, and its avoidance of non-specific staining by the sandwich immunofluorescence technique.

The detection, in patients with pernicious anaemia, of autoantibody against gastric parietal cells has been achieved by immunofluorescence using human stomach, sometimes the patient's own, as substrate (Taylor, Roitt, Doniach, Couchman, and Shapland, 1962; Irvine, 1963). One difficulty of this method for routine testing is the uncertain supply of satisfactory normal human stomach mucosa rich in parietal cells, and another is the non-specific staining of human stomach by the labelled antihuman globulin serum employed in the fluorescent tracing. Evidence has previously been obtained that the reaction of the parietal cell autoantibody may not be species-specific (Abels, de Boer, Jansz, Arends, and Nieweg, 1963): the rat stomach appears to make a satisfactory substrate but confirmation of its reliability in this respect has not so far been reported. The present observations are believed to establish the validity and usefulness of employing rat stomach for immunofluorescence testing of human parietal cell antibody.

METHODS

The general methods of immunofluorescence testing have been described elsewhere (Nairn, 1964).

SERA The sera were obtained from 25 patients with proven or probable Addisonian pernicious anaemia, the diagnostic criterion for which was at least the presence of an endogenous megaloblastic anaemia responsive to treatment with vitamin B₁₂. Forty control sera were from 10 patients with megaloblastic anaemias due to malnutri-

tion, gastrectomy, steatorrhoea, or anticonvulsant drug therapy (see Table II), 10 patients with hypochromic anaemia, 10 non-anaemic hospital patients, and 10 normal subjects, either medical students or members of the laboratory staff.

RAT STOMACH Rat stomach blocks, 4 × 2 × 2 mm., from the whole thickness of the wall at the proximal part of the glandular zone, were obtained from young adult albino animals of either sex. They were snap frozen at -68°C. and stored at this temperature for periods of up to two weeks until used for the preparation of fresh frozen sections which were cut in a cryostat at -20°C. Sections (6μ), mounted on chemically cleaned glass slides without any slide adhesive, were dried in a current of air at 2°C. and were used for fluorescent staining within 24 hours of cutting.

STAINING Immunofluorescent staining was carried out by the sandwich method in which the sections were first treated for 30 minutes with a drop of undiluted test serum at room temperature in a damp atmosphere. After rinsing and washing twice, for five minutes each time, in phosphate-buffered saline (pH 7.1, 0.01M phosphate), the preparations were partly dried in a stream of cold air for five minutes and then treated for 20 minutes with anti-human-globulin labelled with lissamine rhodamine B (RB 200). They were again rinsed and washed in buffered saline, mounted in buffered glycerol, and examined by bright-ground fluorescence microscopy using ultraviolet-blue illumination. The use of clean untreated slides and the brief drying step immediately before applying the conjugate help to prevent sections becoming detached during the final washing. Non-specific staining of sections was minimized by extraction of the conjugate with powdered activated charcoal followed by four

successive absorptions respectively with rat and pig stomach powders and homogenates.

The results of the tests in all 65 serum samples were recorded without knowledge of their source, comparisons being made with known negative and positive sera. In 42 tests, also conducted 'blindly', the staining reactions with human stomach were examined using snap-frozen blocks of mucosa from the most proximal region of extensive gastrectomy specimens. Preliminary conventional staining of frozen sections by the Paragon Multiple Stain (Paragon C. & C. Co. Inc., New York) provided a rapid convenient method of demonstrating the presence of parietal cells, which should be abundant for satisfactory immunofluorescence testing.

RESULTS

Of the 25 sera from pernicious anaemia patients, 22 contained circulating antibodies against parietal cells of rat stomach (Fig. 1). The clinico-pathological data, which included sex, age, serum vitamin B₁₂ level and, in a proportion of cases, Schilling's test and gastric acidity, could not be correlated with the variations observed in immunofluorescent staining, nor with the negative findings in three cases (Table I). The results of the 18 tests on human stomach (Fig. 2) showed close correlation with the rat stomach staining. The only variation observed was slightly stronger staining of the rat stomach in six cases.

The megaloblastic anaemia sera other than per-

FIG. 1. Rat gastric mucosa treated with pernicious anaemia serum (case 25, Table I) followed by RB200-conjugated antihuman globulin. Brilliant (+++) fluorescence of parietal cells $\times 900$.

TABLE I

INCIDENCE OF GASTRIC PARIETAL CELL ANTIBODY IN PERNICIOUS ANAEMIA PATIENTS

No.	Sex	Age (yr.)	Serum B ₁₂ (μ g. per ml.)	Schilling's Test (% of Dose of B ₁₂ Excreted)		Gastric Acidity (Free Acid as mEq./l. after Histamine Administration)	Fluorescent Staining of Parietal Cells	
				Without Intrinsic Factor	With Intrinsic Factor		Rat	Human
1	M	61	<25	...	...	0 ¹	+++	++
2	F	28	30	<1	...	0 ¹	++	+
3	M	61	<25	7.7	...	0	+++	++
4	F	75	30	8.4	...	0	+++	+++
5	F	69	40	<1	9.8	0	++	++
6	F	69	120	7.9	15.5	0	++	++
7	F	64	70	1.5	12.0	...	+++	++
8	F	58	90	5.8	17.0	...	+++	...
9	M	67	50	<1	...	...	-	...
10	F	66	<25	...	...	0	-	-
11	F	82	<25	...	...	0	+	...
12	F	74	30	...	...	0	+++	+++
13	F	80	60	...	...	0	++	...
14	M	72	80	...	...	0	-	-
15	F	72	120	...	...	0	++	++
16	F	77	120	...	...	0	+	...
17	F	79	<25	...	...	...	+++	++
18	M	73	<25	...	...	...	+++	+++
19	F	62	30	...	...	...	++	+
20	M	88	50	...	...	...	++	...
21	F	59	55	...	...	...	++	++
22	F	69	60	...	...	...	+	+
23	F	67	70	...	...	...	++	++
24	M	70	110	...	...	...	+	+
25	F	74	120	...	...	...	+++	+++

¹Gastric biopsy disclosed atrophic gastritis.

FIG. 2. Human gastric mucosa treated as for Figure 1. Brilliant (+++) fluorescence of parietal cells. $\times 900$.

nicious anaemia (Table II) were all negative for circulating parietal cell antibodies when tested on either rat or human stomach. One only of the 10 serum samples from patients with hypochromic anaemia showed a positive reaction with parietal cells of rat and human stomach. The sera from the non-anaemic subjects were all negative with rat stomach, and, in the cases examined, negative also with human stomach (Table III).

TABLE III
INCIDENCE OF GASTRIC Parietal Cell Antibody in Hypochromic Anaemias, Non-Anaemic Hospital Patients, and Normal Subjects

	Fluorescent Staining of Parietal Cells	
	Rat (proportion of cases)	Human
Hypochromic anaemias	1/10 ¹	1/7 ¹
Non-anaemic hospital patients	0/10	0/6
Normal subjects	0/10	0/6

¹Same case. Fluorescent staining (+++) both with rat and human stomach.

DISCUSSION

The results demonstrate that for the detection of antibodies to gastric parietal cells by immunofluorescence the rat stomach provides a substrate at least as good as human stomach. In all 42 cases examined with both methods there was complete correspondence in results apart from brighter staining of rat parietal cells by a few sera. This advantage of the rat stomach could be attributable to the ease with which fresh material can be obtained for sectioning.

A reliable and simple method for detecting antibodies to gastric parietal cells is likely to be extremely valuable in epidemiological studies of pernicious anaemia (te Velde, Abels, Anders, Arends, Hoedemaeker, and Nieweg, 1964) and could help in the detection of early clinical or subclinical cases. Our uniform negative findings in non-pernicious megaloblastic anaemias may foreshadow a useful diagnostic role here. Whether the occasional positive reaction in chronic iron-deficiency anaemia also observed by others (Markson and Moore, 1962; Adams, Glen Kennedy, Mackenzie, Morrow, Anderson, Gray and Middleton, 1964) indicates any closer relation

TABLE II
INCIDENCE OF GASTRIC Parietal Cell Antibody in Patients with Megaloblastic Anaemias Other than Pernicious Anaemia

No.	Sex	Age (yr.)	Serum B ₁₂ (μ g. per ml.)	Schilling's Test (% of Dose of B ₁₂ Excreted without Intrinsic Factor)	Gastric Acidity (Free Acid as mEq./l. after Histamine Administration)	Fluorescent Staining of Parietal Cells		Clinical Condition
						Rat	Human	
1	F	68	30	3	0	—	—	Subtotal gastrectomy for carcinoma
2	M	81	270	4.1	0	—	—	Malabsorption syndrome
3	F	73	300	27.0	0	—	...	Malnutrition
4	M	43	50	7.3	80	—	—	Dilantin treatment of epilepsy
5	F	52	35	...	0	—	—	Malnutrition
6	M	66	125	...	0	—	...	Partial gastrectomy for duodenal ulcer
7	M	79	170	...	...	—	—	Duodenal ulcer and malnutrition
8	M	64	190	...	...	—	...	Chronic pancreatitis and steatorrhea
9	M	92	200	...	...	—	—	Rectal carcinoma and malnutrition
10	F	68	600	...	...	—	...	Malnutrition

ship to pernicious anaemia than the mere sharing of chronic gastritis remains to be determined.

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REFERENCES

- Abels, J., de Boer, W. G. R. M., Jansz, A., Arends, A., and Nieweg, H. O. (1963). *Ned. T. Geneesk.*, **107**, 2074.
- Adams, J. F., Glen, A. I. M., Kennedy, B. H., Mackenzie, I. L., Morrow, J. M., Anderson, J. R., Gray, K. G., and Middleton, D. G. (1964). *Lancet*, **1**, 401.
- Irvine, W. J. (1963). *Quart. J. exp. Physiol.*, **48**, 427.
- Markson, J. I., and Moore, J. M. (1962). *Lancet*, **2**, 1240.
- Nairn, R. C. (1964). *Fluorescent Protein Tracing*, 2nd ed. Livingstone, Edinburgh and London.
- Taylor, K. B., Roitt, I. M., Doniach, D., Couchman, K. G., and Shapland, C. (1962). *Brit. med. J.*, **2**, 1347.
- te Velde, K., Abels, J., Anders, G. J. P. A., Arends, A., Hoedemaeker, Ph. J., and Nieweg, H. O. (1964). *J. Lab. clin. Med.*, **64**, 177.

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