given 550-600 r (13.7 r per minute) in a single session. The depression of all cellular elements was more severe in our series than in that of Riopelle, Ades and Morgan(4) who irradiated 4 monkeys with 350 r (17 r per minute). Our results show certain marked similarities to those of Haigh and Paterson (5) who irradiated 50 monkeys with 260 r, 28 with 500-550 r, and 11 with 600 r (all at 3 r per minute). The effect of 450 r on erythrocytes, eosinophiles and lymphocytes in our monkeys was much like that observed after their intermediate dose, 500-550 r. The response of neutrophiles was also similar except that the secondary elevation appeared to begin earlier in our study. Haigh and Paterson(5) observed peak neutrophile counts on the 26th and 41st days after 500-550 r, and attributed this phenomenon to "a consistent and exact duration of inhibition of precursor cells, related to dose." An almost identical picture was observed in our monkeys in that peak neutrophile and total leukocyte counts were seen consistently on the 26th and 40th days after 450 r (Table I). The secondary elevation of monocytes to supernormal levels observed by Haigh and Paterson(5) was not noted in our series.

Summary. Total body x-irradiation of monkeys with 450 r resulted in significant depression of leukocytes as early as second day with maximal effect noted between 10-17 days. Erythrocytes were affected at a later interval with maximum depression at 17-19 days post-irradiation. Recovery of leukocytes and erythrocytes to normal levels was observed between 3-3½ months.

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1. Schlumberger, H. G., Vazques, J. J., *Am. J. Path.*, 1954, v30, 1013.

2. Saslaw, S., Carlisle, H. N., *J. Lab. Clin. Med.*, 1959, v53, 896.

3. Eldred, E., *Blood*, 1959, v14, 187.

4. Riopelle, A. J., Ades, H. W., Morgan, T. E., *Rad. Res.*, 1957, v7, 581.

5. Haigh, M. V., Paterson, E., *Brit. J. Radiol.*, 1956, v29, 148.

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Electrophoretic Fractionation of B_{12}-Binders in Gastric Juice from Patients with Pernicious Anemia and from Controls.* (26009)

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The intrinsic factor (I.F.) of gastric juice has not been chemically isolated. There is some evidence that it may be associated with B_{12}-binding(1-4), although several B_{12}-binders without I.F. activity have been isolated (5-8), as have substances with high I.F. activity and a remarkably low B_{12}-binding capacity(9,10). However, whether or not I.F. actually binds Vit. B_{12}, it seems to be intimately associated with a B_{12}-binder in gastric juice(4), which may accordingly be used to trace I.F. The present study compares B_{12}-binders in gastric juice from pernicious anemia patients with those from controls.

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Methods. Human gastric juice was collected by a previously described method, with *in vivo* neutralization of the acid juice to prevent peptic autodigestion(11). Precautions were taken to avoid extragastric contaminants. The fasting secretion as well as the histamine, carbamylcholine chloride, and insulin-stimulated secretions were investigated. Within 3 hours after collection, $B_{12}Co^{60}$ was added to the juice. In some cases total B_{12}-binding capacity of gastric juice was estimated by a dialysis technic(12). In these cases, amount of Vit. B_{12} added for electrophoresis is expressed as percent of total binding capacity (Tables I, II). Following concentration and ultrafiltration through collodion membrane for about 24 hours at +4°C,

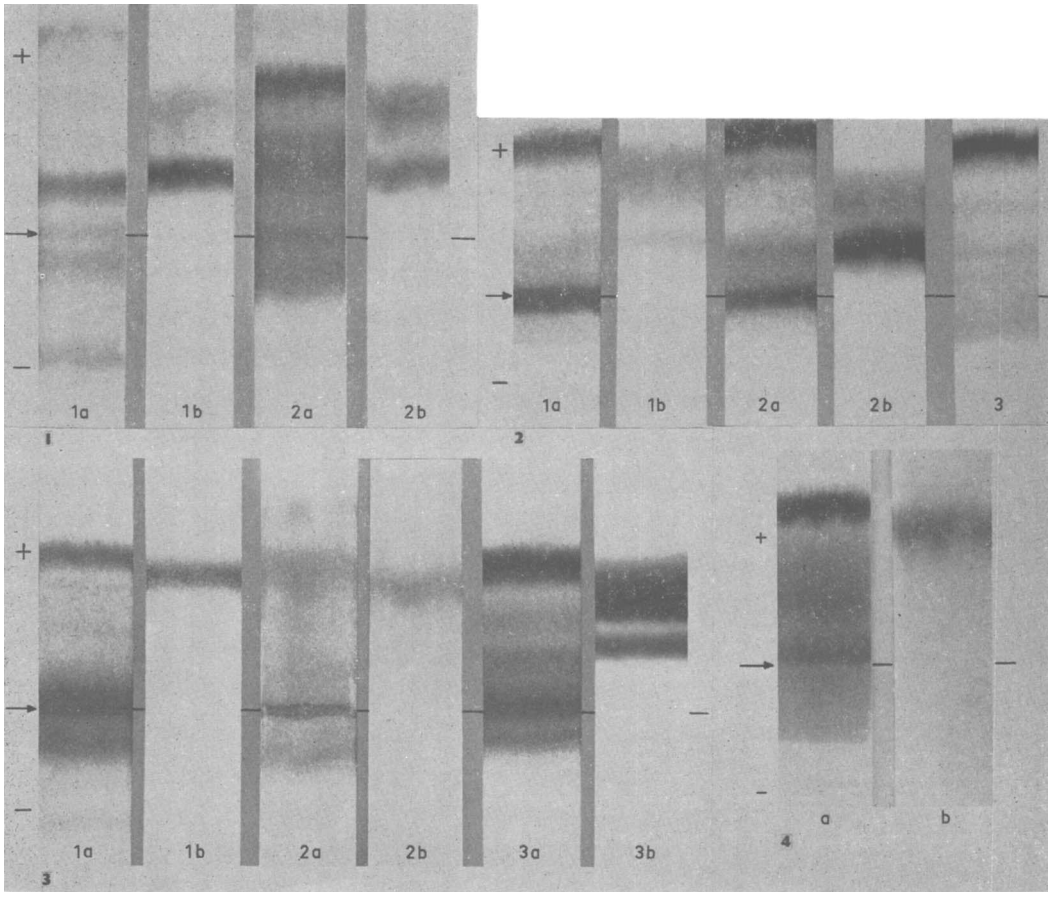

FIG. 1. Acid gastric juice. 1a. Gastric juice of pH 3, not neutralized. Paper electrophoretic strip. Amido black stain. 1b. Autoradiogram of 1a. Two B_{12}-binders are present. 2a. Same individual. Paper electrophoretic strip of gastric juice neutralized in stomach to pH 7.2. Amido black stain. 2b. Autoradiogram of 2a. Two B_{12}-binders are present.

FIG. 2. Gastric juice from patient with histamine-fast achlorhydria and normal Schilling test (Table I, case 7). 1a. Fasting secretion. Paper electrophoretic strip. Amido black stain. 1b. Autoradiogram of 1a. Two B_{12}-binders are present, but slower component is only just visible. 2a. Carbamylcholine chloride stimulation. Paper electrophoretic strip of gastric juice. Amido black stain. 2b. Autoradiogram of 2a. Compared with fasting secretion, there is a relative increase of slower component. 3. Paper electrophoretic strip of diluted serum to show localization of albumin. Amido black stain.

FIG. 3. Gastric juice from patient with histamine-fast achlorhydria and normal Schilling test (Table I, case 8). 1a. Fasting secretion. Paper electrophoretic strip. Amido black stain. 1b. Autoradiogram of 1a. Slower migrating B_{12}-binder is absent. 2a. Same juice as 1a and 1b, incubated with crystalline pepsin at pH 2 at 37°C for 30 min. before addition of vit B_{12}. Paper electrophoretic strip. Amido black stain. 2b. Autoradiogram of 2a showing no appreciable change of B_{12}-binder. 3a. Carbamylcholine chloride stimulation. Paper electrophoretic strip of gastric juice. Amido black stain. 3b. Autoradiogram of 3a. Two B_{12}-binders are present.

FIG. 4. Gastric juice from patient with pernicious anemia (Table II, case 5). a. Carbamylcholine Cl stimulation. Paper electrophoretic strip of gastric juice. Amido black stain. b. Autoradiogram of a. Slower migrating B_{12}-binder is absent.

the juice was subjected to paper electrophoresis in borate buffer of pH 9.0 and ionic strength of 0.12(13). Kodak Kodirex film was used for autoradiography, with exposure times of 4 to 7 days. The paper strips were placed on the film unstained, since bound B_{12} is released on heat-drying of the strips(1,14) and is then washed out during the staining procedure. Since it has been shown that peptic digestion has different effects on various

TABLE I. B_{12}-Binders in Gastric Juice from Achlorhydria Patients with No Evidence of Pernicious Anemia.

Age	Sex	Histamine-fast achlorhydria	Mode of stimulation of gastric glands	Added B_{12} in % of total binding capacity of gastric juice (%)	Slow migrating B_{12}-binder (? I.F.)	Fast migrating B_{12}-binder	Schilling test; 48 hr urine excretion (%)*
58	♂	+	Fasting		++	+	33
53	♀	+	Fasting		+	++	20
			Carbamylcholine	32	++	+	
71	♀	+	Carbamylcholine	21	+	+	28
55	♂	+	Fasting	2	+	++	50
			Carbamylcholine	4	+	++	
63	♀	†	Carbamylcholine		+	+	34
49	♂	†	Insulin		+	+	17
59	♀	+	Fasting		(+)	+	28
			Carbamylcholine		+	+	
40	♂	+	Histamine	31	—	+	29
			Carbamylcholine	18	+	+	
44	♂	+	Fasting		—	+	28
			Carbamylcholine		+	+	
29	♀	†	Fasting		—	+	39
			Carbamylcholine		+	++	
50	♀	+	Fasting	30	(+)	+	10%
			Carbamylcholine	30	(+)	+	On control 14% With I.F. 51%
71	♂	+	Carbamylcholine		—	+	11% With I.F. 24%

* Normal, $\geq 16\%$; normal-borderline, 11-15%; pathologic, $\leq 10\%$.
† Histamine test not performed.

B_{12}-binders(12), its effect on their electrophoretic mobility was studied. For the B_{12} absorption test a modified Schilling technic with carbamylcholine chloride stimulation was employed(15). *Material.* 1. Fifteen control subjects with hydrochloric acid present in the gastric juice. 2. Twelve patients with gastric achlorhydria (present in 9 even after histamine stimulation), but without evidence of pernicious anemia. The Schilling test was carried out in all twelve. 3. Nine patients with clinically established pernicious anemia. Five of these patients were subjected to the Schilling test with or without I.F.

Results. Two B_{12}-binders were found on electrophoretic fractionation of gastric juice from controls neutralized *in vivo* (Fig. 1). The slower and anodically migrating B_{12}-binder was always present. The faster migrating binder could not be demonstrated in 3 of the 15 cases, and even in the others most of the vitamin was generally bound to the slower component. However, less B_{12} was added than was required to saturate the binders, and it remains to be determined whether the 2 binders have differing affinities for Vit. B_{12}.

Two B_{12}-binders were found also in gastric juice from patients with *achlorhydria* and *normal Schilling test* (Table I, Fig. 2), but here relatively less of the Vit. B_{12} seemed to be bound to the slow component. In 3 cases the slower component was absent in the fasting secretion or in that following histamine stimulation, but appeared following parasympathetic stimulation (carbamylcholine chloride) (Fig. 3). A relative increase of binding to the slower component was noted in other patients following carbamylcholine chloride stimulation (Fig. 2). These observations are of interest in view of the known fact that parasympathetic stimulation augments secretion of I.F.(16). The slower component appeared to be altogether absent in one case. This person had a borderline value in the Schilling test and a normal one in the Schilling

TABLE II. B_{12}-Binders in Gastric Juice from Pernicious Anemia Patients.

Age	Sex	Mode of stimulation of gastric glands	Added B_{12} in % of total binding capacity of gastric juice (%)	Slow migrating B_{12}-binder (? I.F.)	Fast migrating B_{12}-binder	Schilling test; 48 hr urine excretion (%)* Without I.F.	With I.F.
42	♀	Fasting	46	—	+	2	30
37	♀	Fasting	41	—	+	1	19
		Carbamylcholine	55	—	+		†
57	♂	Carbamylcholine	75	—	+	.5	‡
67	♂	"	13	—	+	0	
36	♂	"		—	+	1.5	25
50	♂	"		—	+		
82	♀	"		—	+		
71	♂	"		—	+		
72	♂	Fasting		—	+		

* Normal, ≥ 16%; normal-borderline, 11-15%; pathologic, ≤ 10%.
† 13% with gastric juice from case 4, Table I.
‡ 8% " " " " case 2, Table I.

test with I.F. (Table I, case 12).

In the gastric juice from patients with *pernicious anemia* the slower migrating B_{12}-binder was invariably absent, whereas the faster component was, in all cases, present (Table II, Fig. 4).

Discussion. In normal gastric juice, two B_{12}-binders have been demonstrated electrophoretically earlier but only the slower migrating one seemed to have I.F. activity(3). The present study shows that this B_{12}-binder is absent in gastric juice from pernicious anemia patients. The faster B_{12}-binder migrates somewhat slower than albumin (Fig. 2). It seems to have the same electrophoretic mobility as the main serum B_{12}-binder in the α_1-globulin fraction(17). Gräsbeck's(3) suggestion that this component of gastric juice is a peptic breakdown product is inconsistent with its invariable presence in achlorhydria. Furthermore, peptic digestion did not seem to make significant alteration in electrophoretic pattern of the B_{12}-binders according to some preliminary investigations (Fig. 1 and 3). The method described may well be serviceable for *in vitro* assay of intrinsic factor. It could be of value especially in early diagnosis of pernicious anemia. Deficiency of the slower migrating B_{12}-binder may indicate that the patient will eventually develop pernicious anemia.

Summary. Radiovitamin B_{12} was added *in vitro* to human gastric juice. Paper electrophoresis was done with ultrafiltrated juice, and the strips autoradiographed. Two B_{12}-binders were found. The slower, anodically migrating B_{12}-binder was always present both in normal gastric juice and in gastric juice from patients with histamine-fast achlorhydria and normal Schilling test. It was invariably absent in pernicious anemia, where only the faster component was found. In some achlorhydria cases with normal Schilling test the slower migrating B_{12}-binder was identified following parasympathetic stimulation, but absent in fasting secretion or in that following histamine stimulation. The investigation supports the view that the slower migrating B_{12}-binder observed in electrophoresis of gastric juice is related to intrinsic factor. It is suggested that the method may be of diagnostic value.

1. Ternberg, J. L., Eakin, R. E., *J. Am. Chem. Soc.*, 1949, v71, 3858.
2. Bishop, R. C., Toporek, M., Nelson, N. A., Bethell, F. H., *J. Lab. Clin. Med.*, 1955, v46, 796.
3. Grasbeck, R., *Acta Med. Scand.*, 1956, suppl. 314.
4. Castle, W. B., *Gastroenterology*, 1959, v37, 377.
5. Beerstecher, E., Altgelt, S., *J. Biol. Chem.*, 1951, v31, 189.
6. Chow, B. F., Davis, R. L., Proc. 7th Ann. Meet., Nat. Vit. Found., 1952, p17.
7. Ross, G. I. M., *Nature*, 1950, v166, 270.
8. Gregory, M. E., Holdsworth, E. S., *Biochem. J.*, 1955, v59, 329, 335.
9. Williams, W. L., Ellenbogen, L., Esposito, R. G.,

PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 400.

10. Latner, A. L., *Brit. Med. J.*, 1958, v2, 278.

11. Gullberg, R., Olhagen, B., *Nature*, 1959, v184, 1848.

12. Reizenstein, P., *Acta Med. Scand.*, 1959, v165, 481.

13. Glass, G. B. J., Stephanson, L., Rich, M., *Gastroenterologia*, 1956, v86, 384.

14. Scheid, H. E., Schweigert, B. S., *J. Biol. Chem.*, 1950, v185, 1.

15. Reizenstein, P., *Nord. Med.*, 1959, v61, 65.

16. Mollin, D. L., Booth, C. C., Baker, S. J., *Brit. J. Haemat.*, 1957, v3, 412.

17. Pitney, W. R., Beard, M. F., van Loon, E. J., *J. Biol. Chem.*, 1954, v207, 143.

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Artificial Tumors in Dog Bronchus and Their Implications in Production of Experimental Emphysema.* (26010)

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Early diagnosis of pulmonary tumors by X-ray studies is difficult, and endobronchial lesions often go undetected until they occlude the bronchial lumen with resultant distal atelectasis. The original purpose of this study was to produce lesions in experimental animals that simulate endobronchial neoplasms and to demonstrate these lesions by a bronchographic technic recently described(1). While the bronchographic study was being carried out, it was observed that with increasing time after the lesions were made, some of these animals showed evidence of chronic, progressive pulmonary disease suggesting emphysema. This report presents results of this initial experiment.

Materials and methods. Two methods of producing intrabronchial lesions in animals were tried, (1) ligation of individual bronchi to produce incomplete stenosis, and (2) injection of a plastic material to produce an endobronchial mass which would partially obstruct a bronchus. The first method was ineffective in that the incomplete obstruction produced by constricting ligatures did not result in a demonstrable lesion and could not be effectively studied by X-ray. This method was discarded and the second procedure investigated. To find a satisfactory plastic substance, studies were carried out on various materials. Preliminary observations were made with 4 different types of liquid plastics, which were injected subcutaneously into hamsters. Three of these plastics caused severe inflammatory reactions, followed by varying degrees of necrosis at site of injection. The fourth material, *i.e.*, an acrylic resin emulsion called Rhoplex AC-33, (product of Rohm & Haas Co. of Philadelphia) produced little inflammation or necrosis and apparently acted as a bland foreign body after hardening *in situ*. This material is a milky liquid containing 46% solids and is miscible with water. The Rhoplex was thickened by addition of a sodium polyacrylate, Acrysol G.S., which is supplied by the same company at 12-13% solids in water solution. The latter is extremely viscous and may be handled more easily if diluted with an equal volume of distilled water. When 10 ml of the resulting mixture is added to 20 ml of Rhoplex AC-33, a thick creamy emulsion is obtained, which flows readily through a 25 gauge needle. This preparation may be easily maintained for long periods at room temperature if kept covered. Large dogs were utilized for production of the bronchial lesions. During thoracotomy, a lobar or segmental bronchus was prepared by clearing adjacent connective tissues, and 0.5 to 1.0 ml of the thickened acrylic resin emulsion was injected transbronchially and deposited at the immediate sub-mucosal level. Care was taken not to puncture bronchial mucosa and enter the bronchial lumen. This can read-

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