of 2-ethyl-5-methylbenzimidazole as well as its partial reversal by high levels of Vit. B_{12} varied with seasonal and/or genetic differences. (4) The relative order of lethality of benzimidazoles was the same as in virus inhibitory work, while that of benzenes was the same as the inhibition of microorganisms which synthesize B_{12} and/or riboflavin. (5) The 3 inhibitors which are natural moieties of Vit. B_{12}, methylamide, 1, 2-dimethyl-4, 5-diaminobenzene, and 5, 6-dimethylbenzimidazole, were the least lethal of the compounds tested. The implications of these findings are discussed.

1. Woolley, D. W., *J. Biol. Chem.*, 1944, v152, 225.

2. Boggs, U., Shorb, M. S., *Poultry Sci.*, 1956, v35, 1133.

3. Nakamura, M., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 524.

4. Tamm, I., Folkers, K., Shunk, C. H., Heyl, D., Horsfall, F. L., *J. Exp. Med.*, 1953, v98, 245.

5. Abbott, L. D., Dodson, M. J., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 475.

6. Clayton, C. C., *Fed. Proc.*, 1956, v15, 759.

7. Abbott, L. D., Dodson, M. J., Auvil, D. K., *ibid.*, 1956, v15, 674.

8. Billett, F., *Experimentia*, 1958, v16, 99.

9. Woolley, D. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 745.

10. ———, *J. Exp. Med.*, 1951, v93, 13.

11. Hendlin, D., Soars, M. H., *J. Bact.*, 1951, v62, 633.

12. Tamm, I., Folkers, K., Shunk, C. H., *ibid.*, 1956, v72, 59.

13. Cuthbertson, W. F., Gregory, J., O'Sullivan, P., Pegler, H. F., *Biochem. J.*, 1956, v62, 15.

14. Smith, E. L., Parker, G., *ibid.*, 1956, v62, 14.

15. Fischer, N. A., Benson, E. M., Swendseid, M. E., *Arch. Biochem. Biophys.*, 1958, v74, 458.

16. Landauer, W., *J. Cell. Comp. Physiol.*, 1954, v43, Suppl. 1, 261.

17. Olcese, O., Couch, J. R., Quisenberry, J. H., Pearson, P. B., *J. Nutr.*, 1950, v41, 423.

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Latex-Leptospiral Agglutination Test. (24306)

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The successful use of sensitized latex particles(1) in an agglutination test for rheumatoid arthritis(2) suggested applications in the serodiagnosis of leptospirosis. Latex-leptospiral suspensions were found to form macroscopic specific aggregates with hyperimmune rabbit serum, and the study was extended to the human infection. This report gives details of the technic, and compares results of the latex agglutination test with those obtained in standard agglutination-lysis and microscopic agglutination tests.

Materials and methods. Polystyrene latex particles (diameter, 0.81 μ, in 11% aqueous suspension)* were diluted 1:10 in distilled water, filtered through Whatman #40 paper to remove any aggregates, then adjusted with water until a further 1:100 dilution in glycine-buffered saline solution† had an optical density of 0.25 $\pm$ 0.01 at 650 mμ. The optical density measurements were made in 12 x 75 mm cuvettes, using the Coleman Jr. Spectrophotometer 6A with adapter 6.106. Leptospiral suspensions were prepared from 7-day-old cultures in modified Korthof's medium with yeast extract(3). The organisms were killed by overnight exposure to formalin, which was added to give a final concentration of 0.5%. They were then concentrated by spinning 20 min. at 13,000 G, washed twice with formalinized (0.5%) 0.85% NaCl solution (FS), and resuspended in about 1 ml FS per 100 ml original culture. The concentrate was homogenized, using a tuberculin syringe with 22 gauge needle, and spun 3 min.

* Kindly provided by the Dow Chemical Co., Midland, Mich.

† One liter of aqueous solution contained 10.0 gm NaCl, 7.31 gm NH_2CH_2COOH, and 3.5 ml NaOH (1 N); pH was 8.2 at 25°C.

TABLE I. Standardization of Latex-Leptospiral Antigen.

Leptospiral suspension added*	Reactivity† with: Antiserum diluted 1: 4‡	8	16	32	64	128	Normal sera 1:100 A	B	C	D	E	Saline solution
2.0	4	4	4	3	3	3	1	1	1	—	—	3
1.5	4	4	3	2	1	2	±	±	±	—	—	2
1.0	4	3	2	1	—	—	—	—	—	—	—	—
.8	4	3	2	1	±	—	—	—	—	—	—	—
.6§	4	4	3	2	1§	—	—	—	—	—	—	—
.4	4	4	3	2	1	2	—	±	±	—	—	2
.2	4	3	3	2	2	2	1	1	1	—	—	3
.1	3	2	2	2	1	1	1	1	1	—	—	3

* ml/0.1 ml standardized latex suspension.
† Numerical values represent degree of agglutination; negative sign (—), no reaction.
‡ $\times 10^2$.
§ Optimal volume for diagnostic tests.

at 125 G to sediment any large aggregates. The supernate was adjusted finally with FS to an optical density of 0.60 ± 0.01 at 420 mμ. The optimal concentration for diagnostic tests was determined by adding increasing volumes (0.1 – 2.0 ml) to 0.1 ml aliquants of standardized latex suspension and diluting to 10.0 ml with glycine buffered saline solution (GBS). The resulting antigens were tested against human antisera diluted serially in GBS. Equal volumes (1.0 ml) of serum and antigen were mixed in 12 x 75 mm tubes, the tubes corked, and the contents incubated 90 min. at 56°C. Following incubation the corks were removed and the tubes spun 3 min. at 1000 G. The contents were then examined by transmitted light against a dull black background, the aggregates being kept suspended by briskly tapping the tubes with the fingertips. Degrees of agglutination were recorded in the conventional notation (± to 4+). Table I gives results of a representative antigen standardization. The given serum dilutions take into consideration the final 2-fold dilution by antigen. Sera of healthy individuals, who served as controls, were tested at the lowest dilution (1:100) used for diagnostic tests. Excess or deficiency of leptospiral suspension predisposed to spontaneous agglutination, as well as to pseudo-reactions with normal sera. However, there was an intermediate specific zone in which reactivity varied inversely with volume of leptospiral suspension. Optimal volume was the smallest in this range; ordinarily it approximated 0.5 ml. Larger volumes of latex-leptospiral antigen prepared for diagnostic tests contained the components in the same proportion. Freshly-prepared latex-leptospiral antigen was visibly particulate, but appeared less definitely so when diluted with test serum. Preliminary "aging" reduced the particulate appearance, the antigen becoming stabilized over a period of 10-14 days, and remaining constant in reactivity for at least 2 years. The pool of leptospirae used in preparing latex antigen for qualitative screen tests contained *L. icterohemorrhagiae, L. pomona,* and *L. canicola* suspensions in equal volume. These were the serotypes most frequently encountered in our own serodiagnostic practice, and as a pool exhibited a high degree of cross reactivity with antisera representing 17 other serotypes. Sera used in evaluating sensitivity of the tests fell into 2 categories; 1) paired specimens from patients with leptospiral infections verified by isolation and identification of the inciting agent, and 2) single specimens from patients with clinical leptospirosis confirmed by serologic tests. The former were obtained through Miss C. McComb‡ from Dr. J. T. Tonge§, who provided ancillary data on the inciting serotypes, days of illness, and agglutination-lysis titers. The latter were encountered in serodiagnostic practice, and were subjected to microscopic agglutination tests by Dr. N. Hirschberg.‖ The indispensable and generous

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TABLE II. Comparison of Latex-Agglutination (L-A) Serum Reactions with Agglutination-Lysis (A-L) or Microscopic-Agglutination (M-A) Reactions in Human Leptospirosis.

Leptospira isolated	Days ill	Serum reactions: Quantitative* A-L	Quantitative* L-A	L-A screen†
Australis A	2	—	—	—
	39	300	—	—
	3	—	—	—
	38	3,000	1,600	R
	5	—		—
	82	1,000	1,600	—(R)
	4		400	—(R)
	86	100	200	R (R)
	2	—	400	—(R)
	83	300	100	—(R)
	4	—		
	25	3,000	12,800	
	45	3,000	12,800	R
	3	—	—	—
	75	300	—	R
Australis B	7			—
	44	100	1,600	R
	3	—	100	—
	22	300	25,600	R
	3	—		—
	72	1,000		—
	3	—		—
	41	300	200	R
	5	—	400	—
	52	1,000	3,200	R
	1	—		—
	46	300	200	R
	4	—	400	—(—)
	53	100	400	—(R)
	3	—		—
	67	100	400	R
	4	—	800	—(R)
	23	300	800	R (R)

Leptospira isolated‡	Days ill	Serum reactions: Quantitative* A-L	Quantitative* L-A	L-A screen†
"Kremastos"	2			—
	32			R
"	3			—
	57	100		R
hyos	4			—
	52	300	6,400	R
pomona§	8	—	100	R
	21	1,000	3,200	R
" §	6	—	200	—
	22	1,000	3,200	R
" §	6	—	—	—
	22	1,000	1,600	R
"	2	—	—	R
	45	300	800	R
"	2	—	—	—
	40	100	800	R
"Robinson"	3	—		—
	21	10		R
		Quantitative M-A		
bataviae§				
	41	1,600	3,200	R
canicola§				
	32	6,400	12,800	R
ictero.§				
	44	6,400	12,800	R
pomona§				
	77	3,200	6,400	R

* Titers are reciprocals of highest dilutions reacting (2+ to 4+) with respective homologous antigens.

† Reactive (R = 2+ to 4+) or non-reactive (negative sign = ± to 1+) at a final 1:100 dilution with an antigen in which leptospiral component was pooled *L. ictero., L. pomona,* and *L. canicola.* Results in parentheses represent repeat tests in which *L. canicola* was replaced with homologous leptospirae.

‡ Except in cases designated § (q.v.).

§ Leptospirae not isolated; diagnosis based on serologic tests.

contributions of these collaborators are gratefully acknowledged. The control sera used in evaluating the specificity of the latex tests were from healthy individuals, and from patients with syphilis. All were stored at –20°C. Serial 2-fold dilutions in GBS, beginning at 1:50, were prepared for quantitative tests with homologous antigen, and a 1:50 dilution served for qualitative screen tests with pooled antigen. The testing procedure has been described under antigen standardization. Sera were designated reactive (2+ to 4+ agglutination) or non-reactive (– to 1+ agglutination). Titer was expressed as the reciprocal of the highest reactive dilution; numerical value, as in the agglutination-lysis and micro-

scopic-agglutination tests, took into account the final 2-fold dilution of serum by antigen.

Results. Results obtained in quantitative latex-agglutination (L-A) tests of human sera are compared in Table II with those of agglutination-lysis (A-L) and microscopic-agglutination (M-A) tests conducted in other laboratories; 30 individuals are represented. L-A titers with few exceptions were higher than those of standard A-L or M-A tests, the greater sensitivity of the test in some instances making the difference between reactivity and non-reactivity, and revealing antibody before it was detectable by standard procedures. A qualitative L-A screen test in which the antigen component was pooled *L. icterohemorrhagiae, L. pomona,* and *L. canicola* was evaluated with the same sera. Although the component leptospirae were present at one-third the optimal concentration for quantitative tests, reactions(R) were observed in all 8 cases of homologous infection. Reactivity was also observed in 16 (76%) of 21 patients whose infections were caused by other leptospiral serotypes such as *australis* A or B, "Kremastos"(4), *hyos,* "Robinson"(4), and *bataviae.* The screen test failed to detect antibody in 5 of 16 cases of *australis* A or B infection. The initial negative result, however, was reversed in 3 instances by replacing the *L. canicola* component of the antigen with homologous leptospirae. There was insufficient serum to retest the others. The specificity of the latex agglutination reaction was examined in tests of 162 healthy and 14 syphilitic individuals. Three of the former reacted, but only at the lowest final dilution (1:100) tested; the remainder were seronegative. The latex-agglutination tests thus compared favorably in sensitivity and specificity with standard agglutination-lysis and microscopic-agglutination tests. They had additional advantages in simplicity and rapidity of performance, and in the use of a stable, noninfectious antigen.

Summary. The reaction of leptospiral antisera with an antigen composed of polystyrene latex particles and formalin-killed leptospirae provides the basis for a simple, macroscopic tube agglutination test for leptospirosis.

1. Singer, J. M., Plotz, C. M., *Am. J. Med.,* 1956, v21, 888.

2. Plotz, C. M., Singer, J. M., *ibid.,* 893.

3. Borg-Petersen, C., Fagraeus, A., *Acta Path. et Microbiol. Scand.,* 1949, v26, 555.

4. Smith, D. J. W., Brown, H. E., Tonge, J. T., Sinnamon, C. N., MacDonald, V. M., Ross, C. J., Doherty, R. L. *Australasian Ann. Med.,* 1954, v3, 98.

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Gas-Liquid Chromatography of Highly Unsaturated Fatty Acid Methyl Esters.* (24307)

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Biochemical studies of fatty acid metabolism have been given great impetus by the development of gas-liquid chromatography (GLC) by James and Martin(1). This method permits microgram mixtures of methyl esters of fatty acids to be resolved when these esters are volatilized and distributed between a moving carrier gas (nitrogen or argon) and a stationary liquid phase. At the high temperatures (up to 200°C or more) required for volatilization, degradation of saturated fatty acid esters has not been encountered, but the possibility that the double bond structure of highly *un*saturated esters might be altered under these conditions has not been ruled out. In view of growing interest in the biologic role of unsaturated fatty acids as integral parts of certain enzymes(2) and in human nutrition(3), it seemed imperative to investigate the stability of these acids during

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