

**Title:** Study of Intestinal Immunoglobulins.

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**Objective:** Recent experimental work in other laboratories has shown that external body secretions (e.g. tears, nasal secretions, saliva and intestinal juice) contain immunoglobulins, principally of the IgA type. The role of these globulins is not known at the present time, although antibody activity has been demonstrated in nasal secretions following viral illness in man, and in stool filtrates of cholera patients. The purpose of the work described here is 1) to try to establish the type, and amount, of immunoglobulins in the intestinal juice of Thai subjects and 2) to determine if the normal patterns change in patients during episodes of intestinal diarrhea.

**Description:** Experiments performed to date have been directed at overcoming technical difficulties encountered in the study of intestinal juice immunoglobulins by conventional immunologic methods. The problems which have arisen can be briefly stated:

1. The low levels of immunoglobulins in intestinal secretions require concentration techniques in most cases.
2. The presence of proteolytic enzymes in intestinal secretions results in some degradation of immunoglobulins.
3. Antibody detection techniques sufficiently sensitive to detect antibodies present in low concentrations may not be applicable to intestinal juice, which for example, hemolyzes red cells in hemagglutination studies.
4. Quantitation of immunoglobulins by type (IgG, IgA & IgM) is complicated by the presence of globulin fragments which react with antisera against all the globulins.

**Progress:**

1. Ammonium sulfate precipitates of intestinal juice from normal Thai subjects were resuspended in normal saline, dialyzed and studied for immunoglobulin content. Immunoelectrophoresis shows the presence of IgA as the predominant globulin. A fast moving component is also noted, and reacts individually with all anti-IgG, IgA and IgM goat anti-human sera. The nature of this component is not clear at this time.

The most likely possibility is that it represents a proteolytic breakdown product common to all the immunoglobulins. Notably, the fast component may be absent, or occasionally multiple. This variability be related to how long the globulins have been exposed to enzyme action prior to aspiration of intestinal juice. Ouchterlony plate experiments indicate that the reaction with all antisera is due to the same fragment.

2. Quantitation of immunoglobulins has been performed by the precipitin ring technique (using antibody-agar plates) with purified immunoglobulins as reference standards. Table I shows the quantity of immunoglobulins in the proximal jejunal secretions of one normal Thai subject intubated six times on alternate days. It should be emphasized that the presence of a fragment reactions with all three antisera (see above) may seriously interfere with accurate quantitation of immunoglobulins by the precipitin ring method. The figures in Table 1, therefore, are strictly provisional and preliminary.

3. Search for antibody activities in specimens of intestinal juice from subjects convalescent with diarrhea has been undertaken. Microtitre Hemagglutination Technique. Antigens used in this technique were obtained by NaOH fractionations of all enteric bacteria isolated from the stool and intestinal secretions of each case of acute diarrhea. Since whole intestinal juice and ammonium sulfate globulin fractions of intestinal juice are hemolytic, bile free preparations of globulins were prepared by column chromatography using Sephadex G-25 and DEAE. The preparations obtained were not hemolytic, but contained immunoglobulins in quantities equivalent to whole intestinal juice. By hemagglutination testing, several subjects with shigellosis showed positive results, but the distribution of positive juices over the two week convalescent period was random and did not follow a clear-cut daily pattern. Sera from these patients showed a definite rise in antibody over the two week study period. Interpretation of results so far indicate that other factors in the intestinal juice, aside from its hemolytic properties, may interfere with the hemagglutination test as well.

Immunofluorescent staining-because of difficulties (described above) with the HA method, an indirect immunofluorescent staining technique has recently been used in attempts to demonstrate intestinal juice antibody. Data are not yet available regarding the value of this method.

Table I

Day of study	(mgm % intestinal juice)			Protein concentration intestinal juice (mgm%)	Specific Activity Immunoglobins (mgm Immunoglobins per mgm protein)				Ratios	
	IgG	IgA	IgM		IgG	IgA	IgM	IgA/IgG	IgA/IgM	
1	29	26.5	7.2	305.4	.094	.087	.023	.91	3.68	
3	21.5	31.0	10.0	277.7	.077	.112	.036	1.44	3.10	
5	60	51	13.0	430.4	.139	.118	.030	.85	3.92	
7	24	35	8.7	305.4	.078	.114	.028	1.45	4.02	
9	21.5	41	13.0	333.2	.064	.123	.039	1.90	3.15	
11	16	19	0	347.1	.046	.054	—	1.21	—	

### Mucosal Disaccharidase Assay

Data are not available at this time.

### Xylose Tolerance Test

29 men were studied, and 27 were found normal regarding xylose absorption (greater than 5 gms xylose excreted in 5 hours after ingestion of a 25 gram dose). Of the two subjects who were abnormal, one was restudied a month later and found to have a normal test. The other subject was not restudied. Blood xylose determinations at two hours following ingestion of the sugar correlated fairly well with urine results.

### Neomycin-Xylose "stress" Test

This involves the administration of Neomycin sulfate (two grams in divided doses) during the 24 hours preceding a xylose tolerance test\*.

27 men were tested. Xylose malabsorption occurred in 12 men (less than 5 grams xylose excreted in 5 hours after ingestion of a 25 gram dose), and 5 men had borderline xylose absorption. Of the remaining 11 men, although their xylose absorption was in the normal range, most had less absorption than they had had prior to Neomycin administration.

### Serum Carotene and Vitamin A

24 subjects were studied, and all were normal.

Serum cholesterol, transaminase and alkaline phosphatase were assayed in 24 subjects. Three individuals had slightly elevated alkaline phosphatase values, but other tests were normal.

Serum Total Protein, Globulin and Protein electrophoresis, among 27 individuals studied were all normal with one exception-this subject had a moderately elevated serum globulin level, predominately due to a rise in  $\alpha_2$  globulin.

Following the baseline studies mentioned above the men were divided into two groups one receiving folic acid and the other placebo. The men will be re-studied prior to leaving Thailand.

\* Recent experimental data in Thai subjects show that a small dose of Neomycin Sulfate will, within 24 hours, result in intestinal mucosal damage and xylose malabsorption. The significance of this phenomenon is currently under study to determine if it also occurs in non-Thai subjects. For this reason, the Neomycin-xylose test was performed with the American soldiers described here.