# BLOCKING OF HOG INTRINSIC FACTOR BY HUMAN GASTRIC JUICE AND CERTAIN MUCOPOLYSACCHARIDES, INCLUDING BLOOD GROUP SUBSTANCE *

By ZAIDA CASTRO, VICTOR HERBERT † AND LOUIS R. WASSERMAN

*(From the Department of Hematology, The Mount Sinai Hospital, New York, N. Y.)*

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Intrinsic factor can bind vitamin $B_{12}$ and simultaneously attach to receptors on liver or small intestine in a calcium-dependent manner (1). There appears to be a stoichiometric relationship between the receptors, intrinsic factor and vitamin $B_{12}$ (2).

This report, previously presented in preliminary form (3), is concerned with blocking of the receptor sites for hog intrinsic factor by human gastric juice and certain mucopolysaccharides, including blood group substance.

## MATERIALS AND METHODS

*Gastric juice* was collected before and after insulin stimulation of fasting patients. A Dreiling tube (4) was used to avoid contamination with saliva and bile.[1] Gastric juices were pooled according to the blood group of the donors, centrifuged, neutralized with NaOH (20 per cent), and frozen.

*Saliva* was collected from blood group A-secretors and filtered through gauze.

*Commercial preparations of blood group A and B substances* [2] were obtained from large pools of equine and porcine gastric mucosa after enzymatic digestion and alcohol precipitation.

*Degraded blood group A and B polysaccharides* ("P-1 fraction") were prepared from the commercial preparations (Merck) by mild acid hydrolysis with dilute HCl, pH 2.0, at 100° C for 2 hours, followed by dialysis for 4 days against 4 changes of tap water. This treatment splits off fucose and certain oligosaccharide side chains, some of which determine blood group specificity, but greatly increases cross reactivity with type XIV antipneumococcal horse serum (5, 6).

*Hog intrinsic factor concentrate (HIFC)*, WES 727,[3] was potent in a daily oral dose of 5 mg as measured by Schilling-type testing (7). It contained 15 to 20 per cent of blood group A activity as determined by the isohemagglutination technique (5). Twenty-five $\mu$g of this preparation was almost sufficient to saturate the receptors in a 10 ml aliquot of 10 g of rat liver homogenate suspended in 100 ml of Krebs-Ringer-Tris (KRT) medium (2). Other agents tested were: chondroitin sulfate (General Biochemicals, lot 26752); human chorionic gonadotropin ("Follutein," Squibb); heparin ("Liquaemin sodium," Organon); pneumococcus polysaccharides types I, VII, XIV and XVIII and Friedlander bacillus polysaccharide (obtained from Dr. Ely Perlman, Department of Bacteriology, The Mount Sinai Hospital, N. Y.); dextran, USP, 6 per cent wt/vol in saline (Abbott); hyaluronic acid powder, purity approximately 90 per cent (prepared by Dr. Boaz and provided by Dr. M. Horowitz, Department of Gastroenterology, The Mount Sinai Hospital, N. Y.); radioactive $Co^{60}$-vitamin $B_{12}$ [3] diluted to $10^{-8}$ g per ml, specific activity 1 $\mu$c per $\mu$g, which re-

TABLE I

*Inhibition of hog intrinsic factor concentrate (HIFC) enhancement of liver homogenate uptake of $Co^{60}$-$B_{12}$ by A, B or O pooled human gastric juice (HGJ) or A-secretor saliva* *

| First incubation Added agent | Second incubation Added agent | Counts/ minute | % Inhibition |
|---|---|---|---|
| Experiment A | | | |
| 25 $\mu$g HIFC plus: | | | |
| 1 ml 0.9% NaCl | $Co^{60}$-$B_{12}$ | 2,575 ± 261 | |
| 1 ml A—HGJ | $Co^{60}$-$B_{12}$ | 612 ± 76 | 83 |
| 1 ml B—HGJ | $Co^{60}$-$B_{12}$ | 574 ± 36 | 84 |
| 1 ml O—HGJ | $Co^{60}$-$B_{12}$ | 395 ± 63 | 89 |
| Experiment B | | | |
| 25 $\mu$g HIFC plus: | | | |
| 1 ml 0.9% NaCl | $Co^{60}$-$B_{12}$ | 3,162 ± 182 | |
| 1 ml A-secretor saliva | $Co^{60}$-$B_{12}$ | 615 ± 50 | 81 |

* Vitamin $B_{12}$ content of pooled HGJ and A-secretor saliva [*Euglena gracilis*, Z strain (8); assay kindly performed by Dr. Herman Baker]. A-HGJ = 76 $\mu\mu$g/ml; B-HGJ = 35 $\mu\mu$g/ml; O-HGJ = 66 $\mu\mu$g/ml; A-secretor saliva = 40 $\mu\mu$g/ml. Approximate concentration of blood group substance in HGJ by iso-hemagglutination technique (5): A-HGJ = 70 $\mu$g/ml; B-HGJ = 30 $\mu$g/ml.

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† Present address: Thorndike Memorial Laboratory, Harvard Medical Unit, Boston City Hospital, Boston, Mass.

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TABLE II

*Inhibition of HIFC enhancement of liver uptake of $Co^{60}$-$B_{12}$ by purified A and B blood group polysaccharides*

| First incubation Added agent | Second incubation Added agent | Third incubation Added agent | Counts/minute | % Inhibition |
|---|---|---|---|---|
| Experiment A | | | | |
| 25 μg HIFC plus: | | | | |
| 0.9% NaCl | $Co^{60}$-$B_{12}$ | | 2,876 ± 18 | |
| 25 μg A-bgp | $Co^{60}$-$B_{12}$ | | 1,073 ± 25 | 63 |
| 25 μg B-bgp | $Co^{60}$-$B_{12}$ | | 2,027 ± 16 | 31 |
| Experiment B | | | | |
| 0.9% NaCl | 25 μg HIFC | $Co^{60}$-$B_{12}$ | 2,733 ± 264 | |
| 25 μg A-bgp | 25 μg HIFC | $Co^{60}$-$B_{12}$ | 461 ± 14 | 83 |
| 25 μg B-bgp | 25 μg HIFC | $Co^{60}$-$B_{12}$ | 1,169 ± 25 | 57 |

corded approximately 1 cpm per μμg in our well-type scintillation crystal.

Two, three or four sequential incubations in KRT, each followed by three washes, were performed in two ways: *1*) the agent under study was incubated with liver homogenate *prior* to the addition of HIFC and, *2*) the agent under study and HIFC were mixed together and then incubated with liver homogenate. Otherwise, the protocol was as previously outlined (*2*). After final incubation with $Co^{60}$-$B_{12}$ the radioactivity retained in the washed homogenate was assayed. All samples were run in duplicate. In the tables, the arithmetic mean of the duplicate samples is recorded, ± the actual deviation from the mean.

## RESULTS

The tables record the liver homogenate uptake of $Co^{60}$-$B_{12}$ after pre-incubation with HIFC and the decrease of that uptake recorded as "per cent inhibition" due to the agents tested.

Pooled human gastric juice (HGJ) from A, B, and O individuals and saliva from a blood group A-secretor each diminish the effect of HIFC on the uptake of $Co^{60}$-$B_{12}$ by rat liver homogenate (Table I).

Purified blood group A or B polysaccharides (bgp), either mixed with HIFC in equal concentration and then incubated with liver homogenate (Table II, Experiment A), or added before incubation with HIFC (Experiment B), reduced the effect of HIFC. This reduction was greater when the homogenate was exposed to bgp before exposure to HIFC.

Pooled human gastric juice from blood group A donors (A-HGJ) retains its ability to block the effect of HIFC after heating or dialysis (Table III).

This inhibitory effect of A-HGJ may be almost eliminated by suspending liver homogenate previously incubated with A-HGJ in ethylenediamene-

TABLE III

*Inhibition of HIFC effect on uptake of $Co^{60}$-$B_{12}$ by heated or dialyzed pooled human gastric juice*

| First incubation Added agent | Second incubation Added agent | Counts/minute | % Inhibition |
|---|---|---|---|
| Experiment A | | | |
| Heated* A-HGJ plus 25 μg HIFC | $Co^{60}$-$B_{12}$ | 95 ± 15 | 95 |
| Unheated A-HGJ plus 25 μg HIFC | $Co^{60}$-$B_{12}$ | 63 ± 1 | 97 |
| 0.9% NaCl plus 25 μg HIFC | $Co^{60}$-$B_{12}$ | 1,928 ± 14 | |
| Experiment B | | | |
| Nondialyzed A-HGJ plus 25 μg HIFC | $Co^{60}$-$B_{12}$ | 905 ± 67 | 76 |
| 0.9% NaCl plus 25 μg HIFC | $Co^{60}$-$B_{12}$ | 3,764 ± 152 | |
| Experiment C | | | |
| Dialyzed† A-HGJ plus 25 μg HIFC | $Co^{60}$-$B_{12}$ | 747 ± 0 | 76 |
| 0.9% NaCl plus 25 μg HIFC | $Co^{60}$-$B_{12}$ | 3,095 ± 0 | |

* HGJ heated in boiling water bath at 100° C for 20 minutes.
† HGJ dialyzed at 4°C for 22 hours against 2 changes of 4 L of distilled water.

TABLE IV

*Reversibility with incubation in EDTA or 0.9 per cent NaCl of the inhibitory effect of A blood group-containing pooled gastric juice on the effect of HIFC on liver homogenate $Co^{60}$-$B_{12}$ uptake*

| First incubation Medium, KRT Added agent | Second incubation Suspended in | Third incubation Medium, KRT Added agent | Fourth incubation Medium, KRT Added agent | Counts/minute |
|---|---|---|---|---|
| 1 ml A-HGJ | 10 ml 0.015 EDTA | 25 μg HIFC | $Co^{60}$-$B_{12}$ | 2,539 ± 93 |
| 25 μg HIFC | 10 ml 0.015 EDTA | NaCl | $Co^{60}$-$B_{12}$ | 46 ± 11 |
| NaCl | 10 ml 0.015 EDTA | 25 μg HIFC | $Co^{60}$-$B_{12}$ | 3,187 ± 28 |
| 1 ml A-HGJ | 10 ml 0.9% NaCl | 25 μg HIFC | $Co^{60}$-$B_{12}$ | 2,902 ± 364 |
| 25 μg HIFC | 10 ml 0.9% NaCl | NaCl | $Co^{60}$-$B_{12}$ | 1,423 ± 156 |
| 25 μg HIFC | 10 ml 0.9% NaCl | 25 μg HIFC | $Co^{60}$-$B_{12}$ | 3,525 ± 21 |

tetraacetate (EDTA) or 0.9 per cent NaCl at 37° (Table IV).

When A and B bgp were incubated with liver homogenate without $Ca^{++}$ in the medium (KRT, where $Ca^{++}$ was replaced by an equivalent millimolar concentration of $Na^{+}$) and HIFC was added to a subsequent incubation, the HIFC effect was minimally depressed (Table V). Conversely, with $Ca^{++}$ present in the medium during the pre-incubation with bgp, the effect of subsequently added HIFC was markedly diminished (Table V).

When the homogenate was incubated in either EDTA or 0.9 per cent NaCl after incubation with A or B-bgp or pneumococcus polysaccharide type XIV, there was no inhibition of the effect of subsequently added HIFC (Table VI). The effect of HIFC itself, however, was still marked after incubation in 0.9 per cent NaCl but not after incubation in EDTA (Table VI).

Some dialyzable sugars (fucose, certain oligosaccharide side chains) are released from blood group polysaccharides by mild acid hydrolysis.

TABLE V

*Calcium-dependence of the effect of blood group substance on liver homogenate $Co^{60}$-$B_{12}$ uptake after incubation with HIFC*

| First incubation Medium, KRT Added agent | Second incubation Medium, KRT Added agent | Counts/minute |
|---|---|---|
| 100 μg A-bgp | 25 μg HIFC | 324 ± 1 |
| 100 μg B-bgp | 25 μg HIFC | 678 ± 85 |
| 0.9% NaCl | 25 μg HIFC | 2,836 ± 36 |
| Medium, KRT(-Ca) | | |
| 100 μg A-bgp | 25 μg HIFC | 2,660 ± 30 |
| 100 μg B-bgp | 25 μg HIFC | 2,514 ± 102 |
| 0.9% NaCl | 25 μg HIFC | 3,963 ± 31 |

The nondialyzable residue (P-1 fraction) (5, 6) was resuspended in 0.9 per cent NaCl and tested (as "degraded bgp"). Less degraded bgp was needed to obtain the same "per cent inhibition" of HIFC than was obtained with intact bgp (Table VII). The decrease in $Co^{60}$-$B_{12}$ uptake was proportional to the increased amount of the "inhibitor."

Table VIII records the inhibition by 25 μg aliquots of 16 different polysaccharides of the HIFC effect on the $Co^{60}$-$B_{12}$ uptake of rat liver homogenate. A moderate inhibitory effect of relatively large quantities of Cohn fractions III and IV, and haptoglobin and gastric juice from patients with pernicious anemia, has also been demonstrated.

## DISCUSSION

The ability of hog intrinsic factor concentrate to enhance $Co^{60}$-$B_{12}$ uptake by rat liver homogenate was inhibited *in vitro* by normal human gastric juice from A, B, or O donors, or saliva from an A-secretor. The inhibitory effect remains after heating or dialysis of blood group A human gastric juice. Inhibition is greater when the agent under study is pre-incubated with the liver homogenate before addition of HIFC rather than when it is mixed with HIFC and then added to the homogenate.

This suggests that the inhibitory agents act by attaching to the liver "receptors" for intrinsic factor, thereby blocking attachment of intrinsic factor to the receptors.

The blocking of the intrinsic factor receptors by the various agents studied appears to be calcium-dependent, and reversible by removing $Ca^{++}$ from the system. The various "inhibitors" did not damage the integrity of the liver "receptors"

TABLE VI

*Reversibility with incubation in EDTA or 0.9 per cent NaCl of the inhibition by certain polysaccharides of the effect of HIFC on liver homogenate $Co^{60}$-$B_{12}$ uptake*

| First incubation Medium, KRT Added agent | Second incubation Medium | Third incubation Medium, KRT Added agent | Fourth incubation Medium, KRT Added agent | Counts/minute |
|---|---|---|---|---|
| 25 μg A-bgp | 10 ml 0.015 M EDTA | 25 μg HIFC | $Co^{60}$-$B_{12}$ | 2,627 ± 127 |
| 25 μg B-bgp | 10 ml 0.015 M EDTA | 25 μg HIFC | $Co^{60}$-$B_{12}$ | 2,921 ± 353 |
| 25 μg PnS XIV* | 10 ml 0.015 M EDTA | 25 μg HIFC | $Co^{60}$-$B_{12}$ | 2,752 ± 363 |
| 1 ml 0.9% NaCl | 10 ml 0.015 M EDTA | 25 μg HIFC | $Co^{60}$-$B_{12}$ | 2,468 ± 12 |
| 25 μg HIFC | 10 ml 0.015 M EDTA | 0.9% NaCl | $Co^{60}$-$B_{12}$ | 224 ± 12 |
| 25 μg A-bgp | 10 ml 0.9% NaCl | 25 μg HIFC | $Co^{60}$-$B_{12}$ | 2,320 ± 72 |
| 25 μg B-bgp | 10 ml 0.9% NaCl | 25 μg HIFC | $Co^{60}$-$B_{12}$ | 2,765 ± 173 |
| 25 μg PnS XIV | 10 ml 0.9% NaCl | 25 μg HIFC | $Co^{60}$-$B_{12}$ | 2,816 ± 113 |
| 1 ml 0.9% NaCl | 10 ml 0.9% NaCl | 25 μg HIFC | $Co^{60}$-$B_{12}$ | 2,944 ± 120 |
| 25 μg HIFC | 10 ml 0.9% NaCl | 0.9% NaCl | $Co^{60}$-$B_{12}$ | 1,022 ± 72 |

* Pneumococcus polysaccharide type XIV.

as manifested by the fact that the receptors remained capable of again binding HIFC after removal of the inhibitor. The relative ease of removal of polysaccharide inhibitors as compared with HIFC, on incubation in 0.9 per cent NaCl, suggests a lesser specificity of these agents than of HIFC for liver receptors.

In accordance with the concept of molecular complementarity as the basis of specificity (9), the minimal blocking effect of certain polysaccharides and the strong blocking effect of others suggest that there is a specific moiety of polysaccharide nature involved in the binding of intrinsic factor to liver receptors.

It is possible that this moiety of intrinsic factor consists mainly of terminal β-galactosyl groups. This is suggested by the findings that the most active "blockers" are P-1 fractions prepared from blood group substances and type XIV pneumococcus polysaccharide, all of which react strongly with type XIV antipneumococcal horse serum (5, 6), the reaction appearing related to the presence of terminal β-galactosyl groups (10). The blood group substances themselves are somewhat less effective as blockers than their P-1 fractions, and other pneumococcus polysaccharides studied are less effective than type XIV. The postulated β-galactosyl end-group configuration may not be identical for intrinsic factor derived from different species.

These studies leave intact the concept (11) that the ability to bind to both receptor and $B_{12}$

TABLE VII

*Inhibition of HIFC activity when mixed with intact or degraded blood group polysaccharides*

| Agent mixed with 25 μg HIFC | Amount* | Inhibition |
|---|---|---|
| | μg | % |
| Intact A-bgp | 25 | 74 |
| Intact B-bgp | 25 | 55 |
| Degraded A-bgp ("P-1 fraction") | 25 | 90 |
| Degraded A-bgp ("P-1 fraction") | 10 | 75 |
| Degraded A-bgp ("P-1 fraction") | 5 | 56 |
| Degraded B-bgp ("P-1 fraction") | 25 | 85 |
| Degraded B-bgp ("P-1 fraction") | 10 | 65 |
| Degraded B-bgp ("P-1 fraction") | 5 | 54 |

* Amounts of degraded bgp are stated as the weight of intact bgp from which they were derived.

TABLE VIII

*Inhibition of enhanced liver homogenate $Co^{60}$-$B_{12}$ uptake due to HIFC by polysaccharides*

| Agent* | % Inhibition |
|---|---|
| A-bgp | 74 |
| B-bgp | 55 |
| Degraded A-bgp ("P-1 fraction") | 90 |
| Degraded B-bgp ("P-1 fraction") | 75 |
| Pneum. polysaccharide Type XIV | 65 |
| Pneum. polysaccharide Type I | 11 |
| Pneum. polysaccharide Type XVIII | 8 |
| Pneum. polysaccharide Type VII | 30 |
| Pneum. polysaccharide "C" | 4 |
| Hyaluronic acid | 5 |
| Bacillus polysaccharide Friedlander | 8 |
| Human chorionic gonadotropin | 6 |
| Heparin | 2 |
| Chondroitin sulfate | 13 |
| Dextran | 1 |
| Agar ("Bacto-Agar," Difco; purified†) | 5 |

* Twenty-five μg of agent and of HIFC mixed prior to incubation.

† By Dr. Shaul Kochwa, Dept. of Hematology, The Mount Sinai Hospital, N. Y.

simultaneously may be unique to intrinsic factor. Preliminary experiments with aqueous extracts (12) of consecutive segments of rat small intestine indicate that such extracts may also act to block the intrinsic factor receptor sites on rat liver homogenate. This blocking action diminishes progressively in the more distally located segments and may be related to the presence of decreased quantities of polysaccharides. It has been demonstrated (13) that the average quantity of blood group substance in human intestine is less in extracts made from more distal segments of small intestine. Thus, the greater vitamin $B_{12}$ absorption from ileum (14) than from duodenum and jejunum may be related to a lesser quantity of intestinal receptor blocking materials in the lower small intestine.

The fact that HGJ does not enhance $Co^{60}$-$B_{12}$ uptake by liver homogenate as much as does HIFC suggests that the former may contain a variable amount of substances able to attach to receptors and thereby block intrinsic factor but unable then to bind $B_{12}$. One such substance may be degraded intrinsic factor. This possibility is strengthened by the observation that HGJ, degraded by heat, functions as a blocking agent. The blocking action of degraded or undegraded blood group substance and other compounds secreted into the alimentary tract may constitute the major difficulty in the *in vitro* assay of intrinsic factor in human gastric juice, and may in part explain why such assay is generally reliable only in achlorhydric patients (15), who would have reduced ability to degrade blood group substances and intrinsic factor.

Refractoriness of some patients with pernicious anemia to HIFC therapy has been ascribed to an antigen-antibody reaction; it was suggested (15) that a possible antigen might be blood group A substance, or another contaminant in HIFC, rather than intrinsic factor itself. The present studies indicate that the antigen could be a degradation product of HIFC or of blood group substance, or even type XIV pneumococcus polysaccharide. In this connection it should be noted that P-1 fractions prepared from A substance *are antigenic in A individuals.* Antibody (precipitin) to such fractions does not agglutinate erythrocytes or sensitize red cells to render them agglutinable by Coombs serum (6).

However, refractoriness to HIFC may not be due to antigenicity of hog intrinsic factor since it has been demonstrated that sera of some patients with pernicious anemia, who have never received HIFC, inhibit the activity of HIFC (16). Another factor to consider in the "refractory" case is that the oral administration of a poor $B_{12}$-intrinsic factor product may produce only a minimal hemopoietic response (17).

The question legitimately arises as to the general applicability of data obtained in a heterologous system, such as the present one (HIFC with rat liver homogenate). In this connection the following points should be made. *1*) rat intrinsic factor has been successfully used in place of HIFC in our experimental protocol (*2*). *2*) Species specificity does not appear to play a role in the phase of intrinsic factor action under discussion here. Recent evidence suggests that species specificity is not involved in the attachment of intrinsic factor to receptors, but rather in the subsequent release of vitamin $B_{12}$ from intrinsic factor by a presumed enzyme (12, 18).

These studies may have some relation to the statistical association of pernicious anemia and blood group A, although such relationship is highly speculative (19, 20).

## SUMMARY

The enhancing effect of hog intrinsic factor concentrate (HIFC) on $Co^{60}$-$B_{12}$ uptake by rat liver homogenate receptors may be reduced ("blocked") by blood group substance, degraded blood group substance ("P-1 fractions"), type XIV pneumococcus polysaccharide, human gastric juice (from A, B, or O subjects; undegraded, dialyzed, or heated), and to a lesser extent by other polysaccharides. Blocking is $Ca^{++}$-dependent and EDTA-reversible, as is the effect of intrinsic factor. In accordance with the concept of molecular complementarity as the basis of specificity, it is suggested that intrinsic factor may have terminal $\beta$-galactosyl groups which link to receptors on liver (or small intestine), and that various mucopolysaccharides are effective as blockers of this linkage in proportion to the similarity of their end-group conformation to that of intrinsic factor.

The possibility is raised that human refractoriness to HIFC may be due in part to cross reaction

with antibody to P-1 fractions or type XIV pneumococcus polysaccharide.

## REFERENCES


1. Herbert, V. Studies on the role of intrinsic factor in vitamin $B_{12}$ absorption, transport, and storage. Amer. J. clin. Nutr. 1959, **7**, 433.
2. Herbert, V., Castro, Z., and Wasserman, L. R. Stoichiometric relation between liver-receptor, intrinsic factor and vitamin $B_{12}$. Proc. Soc. exp. Biol. (N.Y.) 1960, **104**, 160.
3. Castro, Z., Herbert, V., and Wasserman, L. R. Inhibition of hog intrinsic factor concentrate (HIFC) effect on $Co^{60}B_{12}$ uptake of liver homogenate by human gastric juice and mucopolysaccharides. Clin. Res. 1960, **8**, 16.
4. Dreiling, D. A. The technique of the secretin test: Normal ranges. J. Mt Sinai Hosp. 1954, **21**, 363.
5. Kabat, E. A. Blood Group Substances—Their Chemistry and Immunochemistry. New York, Academic Press, 1956.
6. Allen, P. Z., and Kabat, E. A. Immunochemical studies on blood groups. XXII. Immunochemical studies on the nondialyzable residue from partially hydrolyzed blood group A, B and O (H) substances (P1 fractions). J. Immunol. 1959, **82**, 340.
7. Ellenbogen, L., and Williams, W. L. Quantitative assay of intrinsic factor activity by urinary excretion of radioactive vitamin $B_{12}$. Blood 1958, **13**, 582.
8. Hutner, S. H., Bach, M. K., and Ross, G. I. M. A sugar-containing basal medium for vitamin $B_{12}$ assay with Euglena; application to body fluids. J. Protozool. 1956, **3**, 101.
9. Wilson, I. B. Molecular complementarity and antidotes for alkylphosphate poisoning. Fed. Proc. 1959, **18**, 752.
10. Allen, P. Z., and Kabat, E. A. Immunochemical studies on blood groups. XXIII. Studies on the cross reactivity of untreated and partially hydrolyzed blood group A, B and O (H) substances with type XIV antipneumococcal horse sera. J. Immunol. 1959, **82**, 358.
11. Herbert, V. Mechanism of intrinsic factor action in everted sacs of rat small intestine. J. clin. Invest. 1959, **38**, 102.
12. Cooper, B. A., and Castle, W. B. Sequential mechanisms in the enhanced absorption of vitamin $B_{12}$ by intrinsic factor in the rat. J. clin. Invest. 1960, **39**, 199.
13. Hartmann, G. Group Antigens in Human Organs. Copenhagen, Munksgaard, 1941.
14. Booth, C. C., and Mollin, D. L. The site of absorption of vitamin $B_{12}$ in man. Lancet 1959, 1, 18.
15. Herbert, V. The Megaloblastic Anemias. New York, Grune and Stratton, 1959.
16. Taylor, K. B. Inhibition of intrinsic factor by pernicious anaemia sera. Lancet 1959, **2**, 106.
17. Skouby, A. P., and Østergaard Kristensen, H. P. Five years' treatment of Addison's anaemia with purified intrinsic factor and vitamin $B_{12}$. Acta med. scand. 1959, **164**, 233.
18. Herbert, V. Mechanism of absorption of vitamin $B_{12}$. Fed. Proc. 1960. In press.
19. Roberts, J. A. F. Some associations between blood groups and disease. Brit. med. Bull. 1959, **15**, 129.
20. Sievers, M. L. Hereditary aspects of gastric secretory function. Race and ABO blood groups in relationship to acid and pepsin production. Amer. J. Med. 1959, **27**, 246.