This review focusses on research performed by the author and coworkers. The absorption, turnover and excretion of cobalamin and the pathogenesis of cobalamin deficiency states are described and the laboratory tests used to diagnose these states are discussed. Topics dealt with in detail include: overall turnover, daily need, enterohepatic circulation and excretion of cobalamin and other corrins. The soluble proteins mediating cobalamin transport and their cellular receptors are described and their nomenclature, isolation, structure and mode of action, the role of calcium in the membrane transport, the evolution of these systems and the analogies with transport systems for other substrates are discussed together with deficiency states, especially fish tapeworm anemia and familial selective vitamin B12 malabsorption with proteinuria. Folate deficiency is a relatively rare cause of megaloblastic anemia in Scandinavia but common in North America and explanations for this difference are suggested. The methods of assaying cobalamin in serum and plasma and the performance of radiovitamin B12 absorption tests are critically evaluated. The measurement of intrinsic factor in gastric juice, serum, amniotic fluid and urine is described.

KEY WORDS: cobalamin, intrinsic factor

The following review mostly describes work performed by myself and my collaborators and emphasizes those aspects of it deemed to be of interest to the academic staffs of clinical laboratories.

I happen to have a scientific pedigree consisting of scientists devoted to the study of megaloblastic anemias whose starting point is fish tapeworm anemia. This disorder was described in 1886 simultaneously by Reyher in Estonia and Runeberg in Finland and is morphologically indistinguishable from pernicious anemia. Until about 15 years ago a large fraction of the population in some regions of Finland used to harbor Diphyllobothrium latum. The spreading of the parasite is due to the North European habit of eating mildly salted, almost raw fish. As only freshwater fish are vectors (the most important one is the pike) (1), it is understandable that the parasite was common in the "land of the thousand lakes".

**Tapeworm anemia and what it teaches**

**An epidemiological study that led to 'reference values'**

The pathogenesis of the condition was explained by von Bonsdorff and his junior collaborators, including myself, who demonstrated that the worm interfered with vitamin B12 (cobalamin, Cbl) absorption thus causing a pure Cbl deficiency in its host (1). In this context a large population study (2, 3) deserves mention, especially since it has profoundly influenced my own scientific development. Thirteen hundred and forty-five persons in a heavily infected area were thoroughly investigated using questionnaires, their stools examined for tapeworm ova, their hematological status assessed, and their serum Cbl concentration assayed microbiologically. About half of the worm carriers had pathologically low Cbl values (2). Also, both in the tapeworm carriers and in those with no tapeworm ova, the serum Cbl concentrations were lognormally distributed (3). Since at that time it was considered to be a law of Nature that serum components had a gaussian distribution, this puzzling observation focussed my interest on distributions and normal values and finally led to the introduction of the reference value concept by myself and Saris (4) in 1969.

**Dependence of hematological values on cobalamin**

Because of the frequency of low Cbl values this study presented an opportunity to examine the dependence of hematological data on the serum Cbl concentration (Figure 1). The red cell count and diameter, and the
MCV and MCH indices correlated with the Cbl concentration so that clear-cut hematological aberrations were noticeable when Cbl was below 75 pmol/L (100 pg/mL). However, the hemoglobin concentration and the hematocrit correlated very poorly with the Cbl level (3). Therefore, the common practise of determining hemoglobin and hematocrit to diagnose pernicious anemia and to evaluate antimegaloblastic drugs must be condemned.

Pathogenesis, disease entity and other central issues of medical philosophy

Fish tapeworm anemia also throws light on the concepts of "pathogenesis" and "disease entity"; the latter is of central importance in clinical laboratory sciences. We who explained the pathogenesis do not doubt that the worm alone is able to cause megaloblastic anemia, especially if it is present in large quantities and for a sufficiently long time, and is located proximally so that it gets to the vitamin before its host (1). However, as pointed out by early critics, there are patients who later in life develop genuine pernicious anemia (apparently their intrinsic factor (IF) production is marginal). Also, insufficient dietary intake of Cbl seems to play a role, because the disease occurred more frequently during the war when animal food was scarce (1).

Thus there is a whole spectrum of pathogenetic mechanisms ranging from conditions caused by the worm alone to dietary deficiency and to situations in which endogenous factors such as lack of IF secretion play a decisive role. In most nutritional deficiency diseases, there is a sum of causative factors, and indeed close scrutiny of other diseases reveals that single pathogenetic causes are rare (5, 6, 7). There are always predisposing and precipitating factors, but their respective impacts differ from one case to another. A disease is not a specific entity like a plant or animal species, it is slightly different in every patient, depending on their genetic make-up, environment, habits, etc. A "diagnosis" is a label chosen for practical purposes to allow communication of the experiences of one expert to another. Clinical chemists need to realise the relative nature of the classification of diseases and the role of laboratory tests in this process of categorization. Also, from a purely theoretical standpoint, one cannot expect any laboratory test to discriminate fully between health and disease.

Overall turnover of cobalamin

Absorption, need and excretion

To understand the genesis of Cbl deficiency it is necessary to know the overall metabolism of this nutrient. Man is dependent on external sources of Cbl and ultimately on its synthesis by microorganisms and its transfer through the nutritional chain. The daily need of Cbl is 0.75–8 nmol (1–11 μg), depending on the criteria of "need" (8). FAO/WHO recommend 1.4 nmol (2 μg) per day (9). When increasing doses of Cbl are given orally, the fraction absorbed decreases rapidly and the amount absorbed approaches a plateau (10). Among the factors that limit the absorption of Cbl taken in physiological quantities are the amount of IF and the number of intestinal receptors for the Cbl-IF complex (8) (these substances will be discussed below). Pernicious anemia patients can absorb 3.7 nmol (5 μg) following a single oral dose of gastric juice (11). Under favorable conditions healthy persons therefore probably absorb more than 7.5 nmol (10 μg) per day. Early research performed by myself in collaboration with Okuda (12, 13) and Reizenstein (14) demonstrated that Cbl is eliminated through the gastrointestinal tract mainly from biliary secretion. Cbl undergoes an entero-hepatic circulation, and IF seems to be needed for its reabsorption (15). The total vitamin B12 content of the body is around 2 μmol (3 mg) (extreme values about 0.75–7.5 μmol). The vitamin occurs in different forms, a small fraction as cyanocobalamin and the rest with other substituents at the site occupied by the cyanide; hydroxocobalamin (or aquo, depending on pH), methyl or 5'-deoxyadenosyl (16), cobalamins with the last two substituents being known to function as coenzymes.

Radioactive turnover studies

In 1958 we demonstrated (14, 17) that when small doses of radioactive Cbl are injected, the radioactivity will mix sufficiently well with endogenous vitamin to permit calculation of the turnover rate of the total body Cbl. Later experiments (18) have confirmed and extended our early results. About 0.1–0.2% of the total body pool is lost per day, corresponding to a loss of 0.75–9 nmol per day (cf. the need). It is truly amazing that the complicated Cbl molecule survives unchanged in the body for several years.

Consequences of slow turnover

The slow turnover has important clinical consequences. Cbl deficiency becomes clinically manifest very slowly, and subclinical deficiency must be common. It is important for the clinical chemist to help to detect such cases. Following cessation of therapy in pernicious anemia, relapse will sometimes be delayed more than five years (17). Both patients and their doctors are tempted to cease therapy, but that may cause irreversible neurological changes including tobacco blindness, infertility, perhaps fetal malformations, etc. Cbl deficiency not only affects synthesis of blood cells but all cells including spermatogenesis. Testicular biopsies of pernicious anemia patients before and after specific treatment show dramatic changes (8).

Role of corrin analogues

Both food and intestinal bacteria contain compounds belonging to the corrin family in addition to Cbl, but their clinical significance is obscure. There is some evidence that serum contains such compounds, and that they may interfere with Cbl determinations, especially if in competitive binding assay the binding protein has an unspecific binding site like the substance which we once named R-protein (19) but now call haptocorrin (HC) as proposed by Nexø and Olesen (20). There is also
Proteins involved in cobalamin transport

INTRINSIC FACTOR BINDS COBALAMIN

The nature of IF remained long obscure, though at an early date it was shown to be a macromolecule, and probably a protein. Following the discovery of vitamin B12, Ternberg and Eakins (24) demonstrated that Cbl preparations had the capacity to render Cbl nondialyzable, and suggested that the binding principle was identical with IF. However, the theory fell into disrepute because it was shown that the Cbl-binding capacity correlated poorly with biological IF activity.

My early work on gastric juice (25) demonstrated that it contained at least two Cbl-binding proteins, one with slow ("S"), the other with rapid ("R") electrophoretic mobility. The IF activity was solely associated with the slowly migrating S-binder whereas the R-protein lacked IF-activity. Simultaneously Bishop et al. (26) showed that when radioactive and nonradioactive Cbl were added in different sequence to gastric juice and assayed for IF using the Schilling test, the vitamin which was first added was preferentially absorbed. These two approaches demonstrated beyond doubt that IF binds Cbl, and subsequently, assay of properly defined Cbl-binding was used as a guide in purifying IF. This in vitro method was a prerequisite for isolating IF because biological testing of numerous chromatographic fractions is impractical. Our group was the first to isolate human IF in 1965–1966 (19, 27, 28).

Later we and others (29–33) developed affinity chromatographic media with Cbl bound to an insoluble matrix to purify IF.

PROPERTIES OF INTRINSIC FACTOR

We found that IF has several interesting molecular properties. Following binding of Cbl the molecule shrinks (22), becomes more resistant to digestion and has a tendency to dimerize (28) and oligomerize (34), and the spectrum of Cbl changes (28, 34). IF has at least two functioning sites, one Cbl-binding site and one receptor-binding site; binding of Cbl to the former apparently has an allosteric effect on the latter (35). Our group was the first to utilize isoelectric focussing following the introduction of the technique by Svensson (now Rilbe) in Sweden. Using amino acids to produce a primitive pH gradient we found the isoelectric point of IF to be around 5 (36). We observed that it was microheterogeneous and consisted of numerous isoproteins (37); apparently variations in the carbohydrates, among them neuraminic acids, are responsible. However, desialylated IF retains its biological activity (38, 39). The microheterogeneity was at that time rather hard to digest and raised the tricky question "what is pure protein?"

HAPTOCORRIN

The other protein in gastric juice, the R-protein or HC, was demonstrated to be ubiquitous and to occur in all body fluids studied (40, 41). It was also found in leukocytes and calculations indicated that leukocytes are the principal and perhaps the only source of this protein in health (40). Its content in plasma increases considerably in chronic myelocytic leukemia, in leukocytosis in general, and sometimes in carcinoma of the liver and breast (see 41, 42). It also increases following coagulation, apparently due to release from leukocytes (43, 44). Elevated levels of plasma HC result in an increased power to bind Cbl and an increased serum Cbl concentration (see 8). Determinations of plasma Cbl and HC are of value in the diagnosis of leukemia and malignancies of other types.

Isoelectric focussing resolves HC into numerous iso-proteins which tend to be more acidic than those of IF (45, 46). The relative contribution of the different iso-proteins varies with the source but the components appear to be roughly the same and not more than two types of HC, myelogenic and secretory, can be distinguished (46) although genetic variants seem to exist (47). The isoproteins of both IF and HC can also be separated using ion exchange chromatography (48). If stepwise changes of buffer are used for elution, packages of isoproteins with somewhat different isoelectric points will be produced. With serum HC, two "proteins" have been distinguished, transcobalamin (TC) I and III (49). There is an abundant literature concerning these proteins and their use in diagnosis. Our group is, however, critical of the justification of calling these packages "proteins" (44). Their concentration probably reflects the capacity of blood or tissue cells to add carbohydrate to proteins. Also, the desialylated isoproteins are quickly cleared from the blood plasma by the hepatic asialoglycoprotein receptor mechanism (21, 41, 50) and their concentration therefore probably reflects the functional capacity of that clearance mechanism.

The physiological function of HC is obscure, but some kind of protective function is assumed (42, 51, 52). Its binding site is much more unspecific than that of IF and in a mixture of IF and HC the latter may be blocked with cobinamide (a corrin compound containing only the planar porphyrin-like structure of Cbl). This is of importance in assaying Cbl with the competitive protein binding technique (53) (vide infra).

The association constant for the binding of Cbl to HC at 37°C is greater than that of IF (54). Therefore, in the gastrointestinal tract Cbl is first bound to HC. The complex is then degraded by pancreatic enzymes and Cbl is transferred to IF (55). In pancreatic insufficiency Cbl is poorly absorbed, though Cbl deficiency has rarely, if ever, been reported. As suggested by us (56), the HC-Cbl complex is not degraded in the absence of pancreatic enzymes (55). Pancreatic insufficiency may be diagnosed by orally administering radioactive Cbl.
bound to HC and observing poor Cbl absorption using the Schilling test (57). Degradation of the Cbl-HC complex may also be assayed in vitro (68).

TRANSCOBALAMIN

In the human body there is a third soluble Cbl transport protein named transcobalamin II by Hall and Finkler (59) and which we and a Danish group (20, 65) prefer to call transcobalamin (TC) only. It occurs in blood plasma and specifically stimulates the uptake of Cbl in extra-gastrointestinal tissues. As suggested by us (60), congenital deficiency of TC causes tissue Cbl deficiency in the newborn (61). The megaloblastosis appears very rapidly following birth, earlier than in other kinds of juvenile Cbl deficiency. (Incidentally, this observation strongly suggests that TC traverses the placenta, and we have some unpublished data on the turnover of labelled TC in rabbits supporting this contention). The disease may be successfully treated by injecting parenterally huge doses of Cbl. In this condition radioactive Cbl is poorly absorbed (20), perhaps due to the TC deficiency (8). This observation and other data support the early suggestion (22) that TC may come from the intestine since many types of cells can synthesize it (20). TC exhibits genetic polymorphism (62, 63).

UPTAKE INTO THE CELL

PERMEASES AND PROTEIN-MEDIATED ENDOCYTOSIS

The soluble Cbl transport proteins may be classified into intrinsic factor, transcobalamin, and haptocorrin (IF, TC, HC) (20, 35, 56, 60, 64). We have called the two first-mentioned proteins mammalian permeases (60). IF is the first known instance of a substance responsible for "protein-mediated endocytosis". Once it was regarded as astonishing that the relatively small Cbl molecule (1355 daltons) had to be bound to a macromolecule in order to be absorbed. However, guided by the philosophy of Leibniz that Nature does not make leaps (natura non facit saltus) (and disregarding quantum physics and mutations which involve tiny jumps) I pointed out that this instance probably represented a common mechanism (22). Today there are numerous instances of such mechanisms: transferrin-induced uptake of iron, lipoprotein-mediated uptake of lipids, the asialoglycoprotein clearance system, etc. Common to most of these systems is that there are specific receptors for the substrates in the plasma membrane of the recipient tissue, that calcium is needed for the binding, and that the protein-substrate complex is taken up by the cell (65). Still, the IF mechanism is the only mechanism of this type which incontrovertibly acts in gastrointestinal absorption of adults.

THE INTRINSIC FACTOR-RECEPTOR

The distal small intestine contains a receptor for the Cbl-IF complex (66) and numerous tissues have a receptor for Cbl-TC (67). During recent years our group has been involved in isolating and characterizing the Cbl-IF-receptor of the brush border of the ileal enterocyte. It can be solubilized with detergent according to Katz and Cooper (68) and isolated using affinity chromatography (69, 70) on a substrate-like medium, IF bound to insolubilized Cbl. The receptor is bound in the presence of Ca$^{2+}$ and at neutral pH, and eluted by chelation of Ca or by acidifying the buffer. Like some other membrane proteins the receptor may also be solubilized from the intestine by proteolysis (71, 72), which cuts off the hydrophilic functional parts from the hydrophobic anchor imbedded in the lipid bilayer. The receptor molecule appears to contain two kinds of subunits, a hydrophilic one, alpha, and a more hydrophobic one, beta, connected with a disulphide bond; the complete receptor probably contains many such subunits (72) (Figure 2). We have also studied the role of calcium in the interaction of Cbl-IF with its receptor and have come to the conclusion that calcium apparently acts on the receptor rather than forming a bridge between sialic acids in IF and in the receptor (73). Most of our experiments have been performed on hog material but the important findings have been verified with human intestine (70).

SELECTIVE MALABSORPTION

Our group has a special reason for being interested in the receptor. In the late 1950's Imerslund (74) in Norway and myself (75) in Finland simultaneously came across a hereditary disease, which I named familial selective vitamin B12 malabsorption with proteinuria (Grasbeck-Imerslund disease or syndrome). This disease becomes manifest when the fetally acquired Cbl stores of children are depleted; i.e. it appears later than TC deficiency. The patients possess the receptor (76),
but whether it is able to perform other functions than to bind the substrate is unknown. It would be interesting to study the detailed structure and functioning of these receptors, but we first need to know how these systems are constructed in healthy man. Using specific antisera and other techniques, we hope to be able to reveal the molecular basis of this disease.

A general view

ANALOGIES, PHYLOGENETIC ASPECTS

Many absorption receptor systems appear to be analogous. Since the structures of heme and Cbl are fairly similar and heme is absorbed in undegraded form, we searched for, and found (77) and have made a preliminary isolation (78) of an intestinal receptor for heme. However, our ideas have led us further. The amino acid composition of the TC receptor isolated from placenta greatly resembles that of TC, and a gene duplication has been suggested as the explanation (79). In considering the immunological cross-reactivities between IF and the alpha subunit of its receptor we arrived at a similar conclusion (70), and the transferrin–transferrin receptor relationship may also be similar (65). When bound to their substrate the soluble transport proteins (i.e. holoproteins) exhibit greater affinity for their receptors than do the apoproteins (65). Peptide mapping and other studies on the Cbl transport proteins suggest that they are all genetically related (20–22, 35, 80). There is also evidence that the different receptors function in a similar way, their hydrophobic parts being fairly similar and calcium usually needed (65).

A GENERAL THEORY

A large number of absorption receptors, and probably other membrane proteins too, have a common evolutionary origin and a similar mode of action; possibly only their substrate-binding parts are truly different. The soluble protein substrates are genetically related to a subunit in their receptors and have retained the tendency of the subunits to aggregate and therefore bind to the receptor by forming a mixed oligomer with the related subunit in the receptor (pseudo- or mixed oligomer theory) (81). In addition to the biochemical arguments I wish to add some clinical evidence: In one of the families with the Gräsbeck-Imerslund syndrome autoimmune phenomena are common. One child of the propositus has vitiligo and proteinuria but no Cbl malabsorption (unpublished observations). I suspect that the common denominator of all these symptoms is an error in membrane proteins; perhaps the genes concerned are adjacent.

It is likely that clinical chemistry will increasingly be involved in analyzing tissues, since the compositions of blood and urine only indirectly reflect the state of affairs in the tissues. The techniques used to study receptors are adaptable to analysis of patient specimens (64).

Humoral transport, cobalamin assay

FROM INTESTINE TO BLOOD

Cbl is transported through the enterocyte in an unknown fashion, mitochondria possibly being involved (82). Energy is clearly required because the absorbed vitamin is able to enter the blood against a concentration gradient (83).

IS IT ACCEPTABLE TO ASSAY TOTAL S-COBALAMIN?

In the blood plasma the bulk of the endogenous vitamin is carried by the ubiquitous HC. However, only the TC-bound form seems to be taken up by the tissues (84). Plasma Cbl is not a homogeneous substance but consists of hydroxocobalamin, traces of cyanocobalamin, and the two coenzyme forms (16, 85); possibly small amounts of other corrins are present (86). Assay of total serum Cbl is therefore analogous to determining protein-bound iodine to evaluate thyroid function. The present situation in the clinical chemistry of Cbl is thus not fully acceptable and research is needed to find out which form of Cbl in plasma correlates best with clinical Cbl deficiency.

WHAT KIND OF ASSAY?

Serum Cbl is today mostly determined using competitive protein binding using IF which has a binding site specific for Cbl. Either pure IF or impure IF with cobinamide-blocked HC may be used. The method gives results lower than when HC is used as the binder and more comparable to those found with the old microbiological assays, especially the Euglena technique (86–89). However, I am not convinced that changing from microbiological assay to competitive protein binding technique, and then to assays with pure IF, has resulted in greater clinical relevance of the results of serum Cbl determinations. In fact, I am inclined to agree that the values obtained with Euglena correlate best with the clinical Cbl deficiency (90).

However, we should be aware that the patients are different today from when the microbiological assays were developed and evaluated. In order to see the full-blown clinical disease states described in textbooks one has to go to the developing countries. In our part of the world patients are usually seen at an early stage of their disease and often they have already received some kind of treatment. It is no longer possible to collect diseased reference individuals of the kind we had 20 years ago. The better discriminatory power of the microbiological assays may perhaps be an illusion.

Combined folate-cobalamin assay

CAUSES OF GEOGRAPHICAL DIFFERENCES

In North America and in Britain folate deficiency is common, and it is my understanding that about half of the cases of megaloblastic anemia in North America are due to folate deficiency. Therefore, it is customary to assay both folate and Cbl on the same serum speci-
men. In Scandinavia the situation is different. Mild folate deficiency is not uncommon, folate determinations are of value in the diagnosis and treatment of malabsorption, and mild nutritional deficiency occurs in the elderly. However, full-blown megaloblastic anemia due to lack of folate is very rare. Therefore folate is seldom routinely assayed together with Cbl. The reason for this geographical difference has frequently been discussed, but little has been printed. At the Second International Symposium on Vitamin B12 and Intrinsic Factor in Hamburg in 1961 I offered the following explanation: As experienced by myself in England during my adolescence and as eloquently ridiculed by the French author Pierre Daninos, in his books on Major Thompson, a true Britisher will never eat anything raw. Boiling, frying and baking vegetables destroys substantial amounts of folate. As pointed out by Victor Herbert (oral statement made in Gothenburg, 1981), beans contain a substance which inhibits folate absorption (91), and Scandinavians eat very few beans. Finally, many English-speaking people either live or have lived in tropical and subtropical areas. It is likely that mild sequelae of tropical sprue are common in this population, and the additive effects of all these factors may explain the prevalence of folate deficiency.

Pernicious anemia, pathogenesis and diagnosis

MULTIFACTORIAL PATHOGENESIS

Pernicious anemia is caused by a failure to secrete IF. That is in turn is mostly due to atrophy of the gastric mucosa, usually caused by an autoimmune process leading to the disappearance of gastric glands and cessation of gastric secretion. The disease is familial (8, 92). It is surprising that many subjects who have serious atrophic gastritis do not suffer from Cbl malabsorption and deficiency. The decisive factor appears to be auto-antibodies against IF which inactivate the small, but apparently sufficient, amounts of IF synthesized (93). Because of the lack of hydrochloric acid secretion there are microorganisms in the upper gastrointestinal tract and, like the fish tapeworm, these bacteria may compete with the host for the vitamin. Finally, many English-speaking people either live or have lived in tropical and subtropical areas. It is likely that mild sequelae of tropical sprue are common in this population, and the additive effects of all these factors may explain the prevalence of folate deficiency.

RADIOACTIVE ABSORPTION TESTS

In the diagnosis of pernicious anemia and in the clinical investigation of gastric diseases it is of interest to study IF secretion and Cbl absorption; the latter is done using radiovitamin B12 absorption tests, e.g. with the Schilling test technique (8, 92). However, the Schilling test is an indirect test which may be influenced by increased retention of Cbl by plasma and tissues, by poor kidney function, etc. A new test deserves mention, the "spot feces test" (94, 95). The patient receives by mouth radioactive Cbl plus radioactive chromium trichloride and a colored substance, e.g. carmine red or nacarate.

Figure 3 — Gel filtration of urine (concentrated about 200 times) together with radioactive cobalamin. The curves correspond to the elution of radioactivity. Following the addition of anti-intrinsic factor IgG (white squares) radioactivity is eluted in the totally excluded volume V0 indicating presence of intrinsic factor that binds cobalamin. As indicated by the arrow, urine contains haptocorrin ("uro-HC"). Black squares: No IgG added. V1, totally excluded volume.

The latter two substances are not absorbed. When the feces become colored a small specimen is collected and counted. Since all 51Cr should be excreted, the Cbl excreted is calculated by multiplying the radio-Cbl counts with the inverse fraction of the 51Cr excreted.

RADIOIMMUNOASSAY OF INTRINSIC FACTOR

The assay of IF in the gastric juice is not very complicated (we like to measure the radio-Cbl binding capacity after blocking of HC with cobinamide) (96), but collection of the juice is unpleasant for the patient and the assay is rarely performed. Therefore there has been an interest in "tubeless gastric analyses". Assay of plasma and urinary pepsinogen may be mentioned as examples. Guided by such considerations we have looked for gastric IF in serum, urine and amniotic fluid. The human Cbl–IF complex was isolated, an antiserum was prepared in a rabbit and used for radioimmunoassay. In spite of the high titre of our antiserum we have not yet detected If in serum, but we have found immunoreactive IF in concentrated urine. Urinary IF could not be detected in most patients with pernicious anemia. There are a few exceptions, possibly cases in which IF antibodies play an important role in the pathogenesis (97). Recently we were able to verify the presence of IF in the urine by demonstrating in gel filtration a radio-Cbl-binding peak with the immunological characteristics of IF (Figure 3). The urinary IF concentration is
about 1/10,000 of that in gastric juice. The RIA was also applied to amniotic fluid taken for various diagnostic purposes and IF concentrations about ten times higher than in urine were detected (98). The highest concentration was observed in a case where the fetus had a malformation preventing the flow of gastric contents into the intestine.

We hope that a properly optimized technique to assay uro-IF will become a simple tool to evaluate gastric function, to diagnose pernicious anemia and to detect this disease at an early stage.

**Concluding remarks**

Of course, to its fans, Cbl is the most important substance in Nature. Our pet substance provides a peeping hole from which we can study how the body and its cells function in health and disease and deduce something about evolution, and it has even led us to contemplate its transport play an important role in clinical laboratories. When properly modified, they should also be useful for the study of other substances.

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