concentrations, up to about 10% (w/w), the equilibrium constant

$$K = \frac{[MeO^-][H_2O]}{[MeOH][HO^-]}$$

can be calculated from the following equation:

$$\frac{[MeOH]}{[H_2O]} \cdot K + 1 = \frac{k}{k_{obs}}$$

where k is the true rate constant, *i.e.*, the same as in aqueous solution, k_{obs} the observed rate constant, and [MeOH] and [H_2O] the molar concentrations of methanol and of water, respectively, in the resin phase. The linear relationship between the values of [MeOH]/[H_2O] and $1/k_{obs}$ was verified by the data obtained from Fig. 1, and the resulting value for K was found to be 11.1. This equilibrium in anion exchangers has thus been determined for the first time.

A detailed description of the procedure as well as of the mathematical treatment will be published in the near future. Investigations on the hydrolysis of ethyl benzoate in water-ethanol mixtures are still in progress. Furthermore, the study of the saponification of several phenolic esters by this method has been started.

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Action of Group Blocking Agents and Enzymes on Intrinsic Factor Preparations

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The binding of vitamin B_{12} appears to be of importance for the physiological action of intrinsic factor [1,2]. It therefore seemed of interest to study the mode of binding of B_{12} to the B_{12}-binding principle contained in intrinsic factor preparations. This was performed by subjecting hog intrinsic factor concentrates and pepsin-inactivated [1] human gastric juice to group blocking agents. During the course of attempts to break down the B_{12}-binding principle with proteolytic enzymes some data were obtained bearing upon the mode of binding of B_{12}, and these results are included in the following together with some data on the physiological activities of the chemically modified preparations.

Vitamin B_{12}-binding was assayed by adding radioactive ^{60}Co labeled B_{12}, whereupon the free and bound vitamin was separated with dialysis [1] or ultrafiltration [3].

The action of the group blocking agents is apparent from Table 1. A clearcut effect is observed with fluorodinitrobenzene, iodine, and monochromatic (280 mμ) ultraviolet light, whereas treatment with *p*-chloromercuribenzoate, acetic anhydride, and nitrous acid lacked effect. The absence of effect of the three last mentioned compounds makes the involvement of SH- and NH_2-groups less likely, whereas the involvement of phenol or imidazole groups seems more probable. An active tyrosinase preparation (Worthington) lacked effect, however. This does not eliminate the possibility of a phenolic group being involved, because tyrosinase has been found to lack effect on tyrosine groups of several proteins [4]. The mode of binding of B_{12} to intrinsic factor preparations seems to be much the same as in the cobalamin protein of sow's milk [3].

When both gastric juice and hog stomach preparations were subjected to thorough digestion with crystalline pepsin, trypsin, and highly purified chymotrypsin and mucinase [5] preparations, addition of B_{12} prior to digestion was found to have a marked protective action against chymotrypsin. A slight similar effect was observed in the case of pepsin, whereas B_{12} did not influence the effect of trypsin and mucinase. The latter were found not to have any effect on B_{12}-binding.

The protective action of B_{12} against chymotrypsin seemed to be limited to very few components of the intrinsic factor preparations. This was shown by digesting two identical samples, one in presence of B_{12}, and the other one in its absence. After digestion, vitamin B_{12} was added to the second sample and both samples

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Table 1. Effect of group blocking agents on B_{12}-binding capacity. GJ = Gastric juice. HS = Hog stomach preparation *.

Treatment before binding assay	Effect on binding capacity
GJ or HS + 32 mg fluorodinitrobenzene/ml, pH 8.6, stirring 3 1/2 h	66 % inactivation
GJ or HS at pH 8.6, alcoholic I_2 solution added to final concentration 2.5×10^{-4} M, afterwards dialysis 21 h to remove I_2	96 % inactivation **
Same, 5×10^{-4} M I_2	beginning inactivation
HS (BFS 209) irradiated 3 h with 280 mμ UV light 4×10^{19} quanta/16 mg, O_2 supplied	25 % inactivation
HS pH 7, 1.7 mg *p*-chloromercuribenzoate/ml	None
HS in half saturated Na-acetate, 0.12 ml acetic anhydride/ml, 0°C, stirring 1 h ***	None
HS in 1 M $NaNO_2$, pH 4.6, 0°C, samples taken up to 82 h	None ****
Tyrosinase 0.2 mg/mg HS, 10^{-6} M $CuCl_2$, pH 6—7, 24 h, O_2 supplied	None

* In all experiments an Organon preparation BFS 209 was used, in many cases also other preparations. BFS 209 bound 0.27 μg B_{12}/mg and was active in the Schilling test [6] in 16.6 mg dose.
** Also complete loss of intrinsic factor activity as observed on two patients.
*** The intensity of the color produced by ninhydrin reaction dropped with 88 %.
**** 10 % inhibition was observed after 82 h. This could be due to direct diazotization of tyrosyl groups.

were dialyzed in order to remove the small molecular material. There was no observable difference in the dry weight of the two dialyzed samples. The possibility that B_{12} had a direct inhibitory action on chymotrypsin was eliminated by the failure to find any inhibition of the chymotryptic digestion of casein by similar concentrations of B_{12}.

A few samples of gastric juice were digested with chymotrypsin with and without B_{12} as described above. Afterwards both the B_{12}-binding capacity and the intrinsic factor activity were assayed, the latter using the urinary radioactivity test [6] employed in previous studies [1]. As is apparent from Fig. 1, B_{12} also protected the intrinsic factor activity. This may indicate that the B_{12}-binder is identical with intrinsic factor or a part of it. The simultaneous loss of B_{12}-binding capacity and intrinsic factor activity after iodination also points in this direction.

The explanation of the protective action of B_{12} against chymotryptic digestion is probably that the cyanocobalamin molecule covers up a chymotrypsin-sensitive bond in the molecule. In view of the above findings with group blocking agents, it is interesting to note that chymotrypsin hydrolyzes, *inter alia*, peptide bonds in which the carbonyl group is supplied by tyrosine [7].

The rate of hydrolysis of the bond which can be protected with B_{12} appears to be slow, because even in the absence of B_{12}

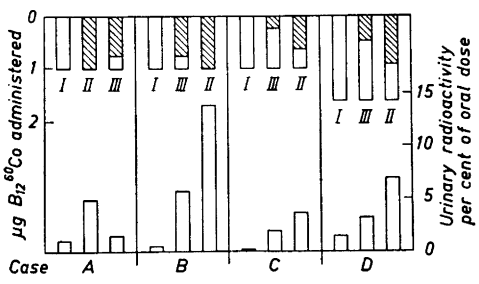

Fig. 1. Intrinsic factor assay of chymotrypsin digested gastric juice. Upper columns: Material administered. White columns: Free B_{12}. Shaded columns: Bound B_{12}. I = Basal test without intrinsic factor, II = Sample digested in presence of B_{12}, III = Sample digested in absence of B_{12}. Lower columns: Patient's response in the Schilling test [6]. *N.B.* In cases A and B sample II was not saturated with B_{12}.

some B_{12}-binding and intrinsic factor activity is retained after 20 h of digestion with high concentrations of chymotrypsin (Fig. 1).

As regards the action of chymotrypsin the present study confirms similar observations made during the preparation of commercial intrinsic factor preparations [8].

Preliminary ultracentrifugal studies [9] indicate that the B_{12}-binder — vitamin B_{12} complex remains a relatively large molecule after trypsin-chymotrypsin digestion (mol.wt. 52 000 both before and after digestion for cobalamin complex from pepsin-inactivated gastric juice). It therefore appears that the B_{12}-binder (and apparently also intrinsic factor) contains only a few bonds sensitive to the action of these enzymes.

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Barium Hydroxide as a Selective Precipitating Agent for Hemicelluloses

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A widely applied method of fractionating and purifying hemicelluloses is by precipitating them as their insoluble copper complexes. The separation of acidic hemicelluloses from neutral ones has been achieved by fractional precipitation using long-chain quaternary ammonium salts (*e. g.* "cetavlon") [1].

Another method has now been found which on its own, or in conjunction with known methods, may enable a more efficient fractionation to be achieved. We have observed that barium ions readily form insoluble complexes with mannans and glucomannans, probably by reaction with the vicinal *cis*-hydroxyl groups on carbon atoms 2 and 3 of the mannose units. Those complexes are precipitated from aqueous solutions on the addition of small quantities of barium hydroxide.

Ivory nut mannan A and spruce glucomannans were both precipitated comple-