fewer deaths occurred among lambs fed diets containing urea than among those receiving cottonseed meal as a source of nitrogen. Observations of calculi in the kidney of 146 of the remaining lambs at slaughter revealed a lower incidence and less calculi in those lambs having received chlortetracycline in the diet. The possibility of a physiological effect of dietary chlortetracycline on the reduction of renal calculi formation and subsequent death from urinary calculi is suggested.

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Effect of Intrinsic Factor Concentrates on Vitamin B_{12} Receptor Protein of Tissues.* (23902)

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Reports from this laboratory indicate that intrinsic factor (IF) containing materials stimulate certain serum proteins to combine with Vit. B_{12}(1) and IF concentrates stimulate uptake of radio-active Vit. B_{12} by slices of rat liver or kidney(2). The data presented here demonstrate that IF containing materials stimulate receptor proteins to combine with Vit. B_{12}. These proteins are distributed in all tissues thus far tested. Furthermore, the data offer support to the view that IF stimulates uptake of Vit. B_{12} in slices by first being absorbed into the cell.

Methods and materials. Wistar strain, adult rats grown on stock diet were used. The animals were decapitated and organs to be studied quickly removed and chilled on cracked ice. Homogenates, 10%, were prepared in buffer by a Potter-Elvejhem homogenizer. Incubations, 1 hr, were carried out in standard Warburg apparatus at 37°. The main chamber contained 1 ml of homogenate, intrinsic factor concentrate (IFC)† dissolved in buffer(3) and buffer to make a volume of 2.6 ml. The side arm contained 0.4 ml of cobalt$_{60}$ labeled Vit. B_{12}‡ in buffer which contained 30 mμg of Vit. B_{12} and approximately 0.05 μc of radioactivity. The final volume was 3 ml. The system was aerated 5 min. with 20% O_2, 5% CO_2, and 75% N_2; after allowing a 5 min. period for temperature equilibration, the vitamin was tipped-in from side arm. The experiment was terminated by aspirating contents of each flask into a pipette and transferring to test tube. To each test tube was added 60 mg charcoal, suspended in one ml of buffer. The charcoal quickly adsorbs the free Vit. B_{12} and stops the reaction. All tubes from each experiment were shaken

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† Intrinsic factor concentrate was supplied by Abbott Laboratories, North Chicago, Ill. Its biological potency was established as 5 mg/day, using urinary excretion test as means of assay.

‡ Radioactive vit. B_{12} was generously supplied by Merck and Co.

TABLE I. Effect of IFC on Amount of Vit. B_{12} Combined by Rat Liver Homogenates.

Conditions	CPM/100 mg of liver	
	Control	100 μg IFC added
Complete incubation mixture*	22,600	22,600
Dialyzed,† incubation mixture (DIM)	6,870	14,700
Supernatant from centrifuged DIM‡	2,700	11,350
Sediment from centrifuged DIM‡	3,995	3,489

* Complete incubation mixture contained 1.0 ml 10% liver homogenate, 30 mμg radioactive B_{12} and buffer or IFC as indicated; final vol 3 ml.

† Dialyzed for 17 hr against 100 vol of isotonic KCl at 5°C.

‡ Centrifuged for 10 min. at 25,000 × g.

5 minutes in a mechanical shaker. These tubes were then centrifuged at 25,000 x g for 10 minutes. Clear supernatant fluid was decanted into cuvettes for assay in a center-well scintillation counter. Data are expressed as counts minute (CPM) corrected for background which was about 600 CPM for our instrument.

Results. It was necessary in preliminary experiments to use dialysis to remove free Vit. B_{12}. In Exp. 2 (Table I) the addition of IFC to the reaction mixture caused a 110% increase in combined Vit. B_{12}. When incubation mixture which contained IFC was cleared of cell nuclei, membranes and mitochrondria by centrifugation, the resulting supernatant fluid contained a 320% increase in combined Vit. B_{12}; whereas the sediment did not show increase. These data demonstrate that IFC stimulates the soluble protein and the microsomes rather than cell nuclei, membranes or mitochondria to combine with Vit. B_{12}. Also, these data support the view that increased uptake of Vit. B_{12}, by tissue slices, reported previously(2), was the result of IFC having been absorbed by the liver slice and thus exerting its stimulatory action intracellularly rather than extracellularly.

It was demonstrated that 75 to 93% of the action of IFC is heat labile, (Exp. 3 and 5, Table II). The disturbing factor, however, in these experiments was the observation that pink cobalamin protein,§ which has no intrinsic factor activity was also potent in stimulating liver homogenates to combine with Vit. B_{12}; this difficulty was resolved in subsequent experiments. The experiments thus far demonstrate that stimulatory action of IFC is associated with soluble proteins of cell or microsomes. This being the case it was no longer necessary to use the cumbersome and tedious dialysis procedure. Thus charcoal was used to remove free Vit. B_{12} and this served to accentuate the stimulatory action of IFC 10-fold, or more, Exp. 5, (Table II). Data in Table III demonstrate that stimulatory action of IFC is operative in both the microsome fraction of liver homogenates and in the soluble portion, to approximately an equal extent.

An extract of liver was prepared by centrifuging a 10% homogenate at 25,000 x g for 15 minutes at 5°, discarding the residue. This extract could be stimulated by IFC to combine Vit. B_{12} (Table IV). The extract was made 30% saturated with $(NH_4)_2SO_4$, and resulting precipitate collected, dissolved in

TABLE II. Effect of Heated IFC, Cobalamin Protein(8) and IFC on Amount of Vit. B_{12} Combined by Rat Liver Homogenates.

Conditions	CPM/100 mg of liver		
	Control	100 μg IFC added	Other additions
Complete incubation mixture*	22,600	22,600	
Dialyzed incubation mixture (DIM)†	8,600	14,510	
DIM	8,600		9,990‡
DIM	8,600		14,230§
DIM, Exp. 2 treated with 50 mg charcoal	405	4,300	
DIM, Exp. 3 treated with 50 mg charcoal	405		690‡
DIM, Exp. 4 treated with 50 mg charcoal	405		4,850§

* Complete incubation mixture contained 1.0 ml 10% liver homogenate, 30 mμg radioactive B_{12} and buffer or IFC as indicated; final vol 3 ml.

† Dialyzed for 17 hr against 100 vol of isotonic KCl.

‡ Intrinsic factor concentrate was dissolved in buffer and heated in a boiling water bath for 30 min. prior to use in these exp.

§ Pink cobalamin protein(8) was added, 100 μg per flask.

§ Pink cobalamin protein was supplied by Dr. K. W. Thompson of Organon Laboratories.

TABLE III. Effect of IFC on Amount of Vit. B_{12} Combined by Soluble Proteins and Microsomes from Rat Liver.

Conditions	CPM/100 mg of liver Control	CPM/100 mg of liver 100 μg IFC added
Complete incubation mixture*	19,000	19,000
Dialyzed incubation mixture (DIM)†	5,500	11,950
DIM, microsomes‡ substituted for liver homogenate	4,950	12,714
DIM, soluble proteins§ substituted for liver homogenate	5,260	12,714
DIM, from Exp. 2, treated with 60 mg of charcoal	302	5,797
DIM, from Exp. 4, treated with 60 mg of charcoal	147	6,745

* Incubation mixture contained 1 ml of 10% liver homogenate, 30 mμg radioactive vit. B_{12} and buffer or IFC as indicated; final vol 3 ml.

† Dialyzed for 17 hr against 100 vol of isotonic KCl.

‡ and § Microsomes were prepared from liver homogenate by first removing mitochondria and other large particles by centrifuging for 5 min. at 30,000 $\times$ g. The microsome fraction was sedimented by centrifugation for 2 hr at 110,000 $\times$ g. The material that resisted centrifugation under these conditions is designated as soluble protein (§) fraction.

water and dialyzed against 100 volumes of isotonic KCl. After dialysis this preparation was not stimulated by IFC to combine with Vit. B_{12}, (Exp. 4, Table IV). Additional $(NH_4)_2SO_4$ was added to make the extract up to 66% saturation. The precipitate which resulted was dissolved in water, dialyzed as above, and contained about 90% of the activity of original dialyzed extract Exp. 5, Table IV. Of considerable interest is the observation that pink cobalamin protein was only 6% as active as IFC, which is in contrast to the observation that it was equally as active as IFC when whole homogenate was used in the incubation mixture. The protein that was soluble in 66% $(NH_4)_2SO_4$ was dialyzed as before and was inactive in this system, (Exp. 7, Table IV).

Data presented in Fig. 1 show effect of concentration of IFC on stimulation of a liver homogenate to combine with Vit. B_{12}. The response is proportional to concentration of IFC added and this response is linear between 10 and 50 μg. No explanation can be offered, at the present time, for the departure from linearity observed when small concentrations of IFC are added. We have not observed this phenomenon when an extract of liver was first purified with $(NH_4)_2SO_4$. Fig. 2 depicts results obtained when neutralized, lyophilized, normal gastric juice was reconstituted and tested with a 10% rat liver homogenate. Under conditions of these experiments gastric juice alone did not bind significant quantities of Vit. B_{12} although it did

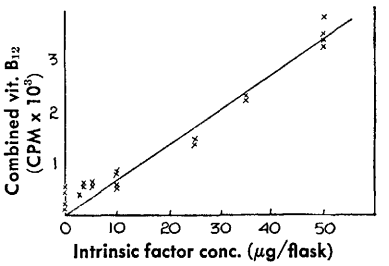

FIG. 1. Combined vit. B_{12} is plotted against the amount of IFC added to each flask. Each flask contained 1.0 ml 10% liver homogenate, 0.4 ml CO^{60}-vit. B_{12}, and 1.6 ml buffer or an appropriate amount of IFC dissolved in buffer.

TABLE IV. Effect of $(NH_4)_2SO_4$ Fractionation of Vit. B_{12} Receptor Proteins from Rat Liver on the Ability of These Fractions to Combine with Vit. B_{12} in Presence or Absence of IFC.

Conditions	CPM/100 mg of liver Control	CPM/100 mg of liver 100 μg IFC added
Complete incubation mixture*	22,800	22,800
Incubation mixture treated with 60 mg of charcoal after incubation (CIM)	100	5,130
CIM, enzyme preparation substituted which had been dialyzed† prior to incubation	100	2,612
CIM, enzyme preparation dialyzed after precipitating with 30% $(NH_4)_2SO_4$ saturation	50	50
CIM, enzyme preparation dialyzed after precipitation between 30-66% $(NH_4)_2SO_4$ saturation	80	2,360
CIM, same as preceding exp. except 100 μg of pink cobalamin protein was substituted for IFC	80	268
CIM, enzyme preparation soluble in 66% $(NH_4)_2SO_4$ and dialyzed‡	63	71

* Incubation mixture contained 1 ml of enzyme preparation, 30 mμg of radioactive vit. B_{12} and buffer or IFC as indicated; final vol 3 ml. The enzyme preparation was a supernatant fluid fraction prepared by centrifuging a 10% liver homogenate for 15 min. at 25,000 $\times$ g.

† Enzyme preparation was dialyzed against 100 vol of isotonic KCl.

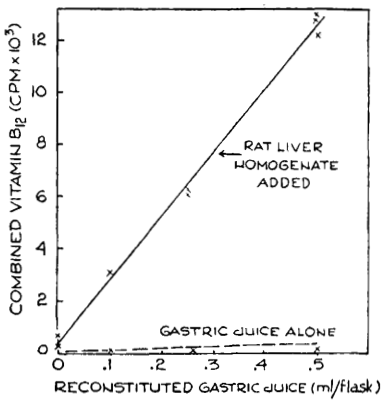

FIG. 2. Combined vit. B_{12} is plotted against the amount of reconstituted gastric juice added to each flask. Each flask contained 1.0 ml liver homogenate, 0.4 ml CO^{60}-vit. B_{12}, an appropriate amount of reconstituted gastric juice, and an additional amount of buffer to make a final vol of 3 ml.

stimulate proteins of liver homogenates to combine Vit. B_{12}.

It was of interest to compare serum proteins and liver homogenates as to their ability to combine with Vit. B_{12} both with and without stimulation of IFC (Fig. 3). It appears that IFC stimulates serum and liver homogenates to combine with Vit. B_{12} to the same extent. One striking difference was observed when one compares the amount of combined Vit. B_{12} without added IFC. About 5 times more Vit. B_{12} was combined by serum than by liver homogenates.

Discussion. Data presented indicate that all of the tissues of the rat, including duodenum, contain receptor proteins which combine with Vit. B_{12} when stimulated by intrinsic factor containing materials. The data reveal that Vit. B_{12} receptor proteins are found in the microsomes and also in the soluble protein fractions of liver homogenates. These B_{12}-receptor proteins are stimulated by reconstituted lyophilized normal human gastric juice and IFC prepared from hog intestinal mucosa. This stimulation is proportional to concentration of added IF containing material. It is of interest that human gastric juice is active in this system whereas it was not active in stimulating uptake of Vit. B_{12} by rat liver slices. These data, together with the reports that human gastric juice(5) hog gastric juice (6) and a commercial hog stomach preparation(7) did not increase the absorption of CO^{60}-B_{12} in the gastrectomized rat, lead us to the tentative conclusion that the active form of intrinsic factor from various species is elaborated in several forms. In order for IF to become available to the host animal the IF concentrate which is secreted or elaborated by the cells of the stomach must be digested in order to present its active site for action on cell surfaces. Should the host animal be unable to do this, the IFC is inactive. We have found that some IF preparations from hog mucosa have varying amounts of activity which stimulate serum receptor proteins to combine with Vit. B_{12}. Some of this activity

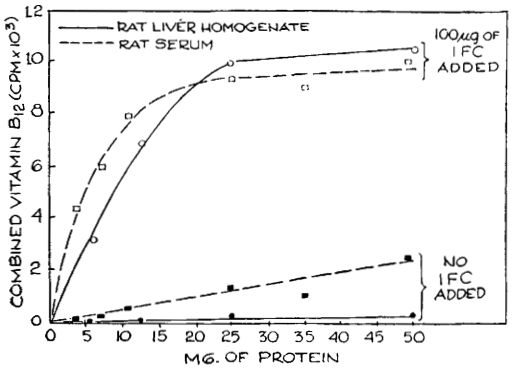

FIG. 3. Combined vit. B_{12} is plotted against mg of protein. Each flask contained 0.4 ml of CO^{60}-vit. B_{12}, an appropriate amount of serum or rat liver homogenate suspended in 2.1 ml of buffer, and 0.5 ml of buffer. 0.5 ml of IFC (200 μg/ml), dissolved in buffer, replaced 0.5 ml of buffer in the experiments indicated.

TABLE V. Comparison of Ability of Homogenates of Various Tissues to Combine with Vit. B_{12} in Presence or Absence of IFC.

Tissue	No. of exps.	CPM/100 mg tissue* Control	100 μg IFC added
None	4		78
Stomach	2	12,148	10,935
Lung	2	463	9,103
Ileum	2	160	8,538
Spleen	4	460	8,479
Skeletal muscle	4	171	7,534
Liver	9	451	7,479
Heart muscle	4	150	6,742
Duodenum	5	356	6,400
Brain	4	75	2,409
Kidney	4	135	1,368

* Incubation mixture contained 1 ml of 10% tissue homogenate, 30 mμg of radioactive vit. B_{12} and buffer or IFC as indicated; final vol 3 ml. After incubation the mixture was treated with 60 mg of charcoal, see text for details.

is not heat labile. However, when these same preparations are treated with crystalline papain all of their activity becomes heat labile.

Although pink cobalamin protein(8) stimulated liver homogenates to combine with Vit. B_{12}, receptor proteins purified by ammonium sulfate fractionation were not stimulated by this curious Vit. B_{12} containing protein. This unexplained phenomenon supports the view that stimulation of purified receptor protein by IFC is a function of its intrinsic factor content. More extensive studies are needed to clarify this point.

A comparison of receptor proteins from serum and liver (homogenates), provides evidence that intrinsic factor may be absorbed into the blood stream of the rat from the intestine. Although other explanations are not precluded, the most plausible explanation of the data shown in Fig. 3 is that rat serum contains a material that acts like IF in the system under investigation. Also, the data presented extend and support a previously stated hypothesis(9,2) that intrinsic factor is absorbed into the blood stream where it acts not only to conserve the dietary Vit. B_{12} by enhancing its combination with receptor proteins of serum but also facilitates absorption of Vit. B_{12} by peripheral tissues by causing it to combine wtih the receptor proteins of the various tissues.

Summary. Data have been presented which show that intrinsic factor containing materials will stimulate receptor proteins to combine Vit. B_{12} of tissues as well as serum. Further, the receptor proteins of tissues are located intracellularly in the microsome and soluble protein fraction of liver homogenates. The evidence suggests that intrinsic factor aids in absorption of Vit. B_{12} by stimulating the intracellular receptor proteins to combine with Vit. B_{12}.

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Competitive Transfer of Sorbose and Glucose in Placenta of Rabbit. (23903)

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Previous studies on the placental transfer of simple sugars have suggested that an active transport mechanism is involved rather than one of simple diffusion(1-5). A differential permeability of the placenta toward the aldo- and keto-hexoses, respectively, has been noted in all species studied (sheep, guinea pig, rat, rabbit, monkey). In at least one of these (monkey) a competitive inhibition of fructose from mother to fetus by excess of glucose in maternal circulation has been suggested(4).

The purpose of the present study was to investigate the possibility of a competitive inhibition of sorbose transfer by glucose in the placenta of the rabbit at term. The findings indicate a significant depression of the "permeability constant" for sorbose in the presence of excess glucose.

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