ous cell culture over mixed cell cultures for studies of chemical inhibition of virus synthesis are discussed. Cellular uniformity permits precise allocation of the observed effects of a test agent to cells capable of propagating the virus.

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Intestinal Absorption of Vitamin B_{12} in Humans as Studied by Isotope Technic.* (21153)

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The intestinal absorption of vit. B_{12} appears to be variable from one individual to another(1-3) and its underlying principles seem to be contradictory and confusing. Only a small part of vit. B_{12} ingested is absorbed in the intestine(4-6) and the hematopoietic responses of patients with pernicious anemia to oral administration of vit. B_{12} even in association with potent sources of intrinsic factor from human or animal origin are, at their best, lower than those observed after parenteral administration of similar doses of vit. B_{12}(1-3,7-10). On the other hand, fecal excretory studies done with radioactive vit. B_{12} tend to indicate that as much as 90-95% might be absorbed when 0.5-1.0 μg is given orally to normal individuals(11-13).

Recently, we have used surface scintillation measurements of the uptake of radioactive vit. B_{12} by the liver following parenteral and oral administration of this vitamin for the study of B_{12} metabolism(14-16). These investigations have shown that in humans under normal conditions the liver is the target organ for the uptake of radioactive vit. B_{12}, especially after its oral administration and that the scanning technic can be successfully applied to evaluate the intestinal absorption of vit. B_{12}.

Method. The measurements were made with the method described previously(16) in 20 normal individuals or patients with irrevalent disorders, following ingestion of standard doses of Co^{60}-containing vit. B_{12}† to which variable doses of crystalline nonradioactive vit. B_{12} were added. Between the 6th and 10th day after ingestion of radioactive vit. B_{12}, when most of the radioactive material had left the gastrointestinal tract, counts were made with scintillation counter (NaI Thallium 1″ x 1″ crystal) in close approximation with the skin at 1000 volts over 3-4 anterior,

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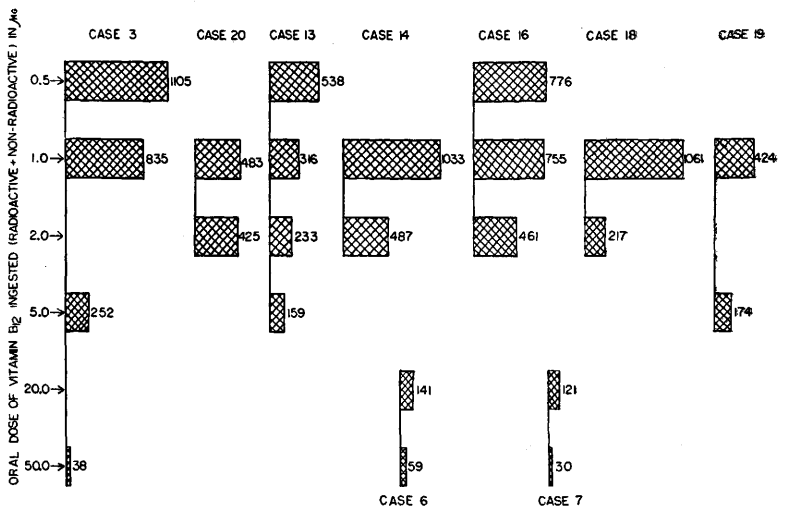

FIG. 1. Averaged hepatic uptake of radioactivity in counts per min. above background in 9 individuals at various oral doses of vit. B_{12} and calculated per 1 microcurie Co^{60}-B_{12} ingested.

antero-lateral, and mid-lateral projections of the liver on 2-4 consecutive days. The counts were measured without amplifier, calculated above background (kept at a low level of about 60 counts per minute), corrected for the efficiency of the scaler with the use of dried Co^{60}-B_{12} standard, and averaged for each day of study. From these multiple averages the mean value of the hepatic uptake was calculated and checked against the mean figure of the averaged abdominal counts which were taken in 6-8 skin projections of the abdominal organs beyond the liver on each day of the study and calculated similarly to the hepatic uptake. These served as a control of the possible scattered radiation emanating from the radioactive material which might still have been retained in the intestines, especially in cases of severe constipation. The mean hepatic counts obtained following ingestion of radioactive B_{12} were then compared with those observed following a similar dose of the vitamin administered parenterally. This allowed one to calculate the parenteral equivalent of the oral dose of vit. B_{12} ingested in the individual tested. Because of the leveling off of the radioactivity counts over the liver during the second week after ingestion of Co^{60}-B_{12}(16) the tests were repeated on the same subjects at intervals of 2-3 weeks with the use of the averaged end counts of the preceding experimental period as the base line for the next experiment.

Results. It soon became evident that there exists an inverse relationship between the radioactivity counts over the liver and the amounts of crystalline vitamin B_{12} added to the radioactive B_{12} ingested. Fig. 1 shows that in the same individual, the uptake of radioactive vit. B_{12} by the liver decreases rapidly on increase of the total dose of vit. B_{12} taken in, when calculated per one μc of radioactive Co^{60}-B_{12} ingested. In view of the law of dilution of radioactive substances in the metabolic pool, this indicates an inverse relationship between the amount of vit. B_{12} ingested and the efficiency of its absorption in the intestine(17). The data in Table I indicate that the oral dose of 0.5 μg B_{12} results in a hepatic uptake of radioactivity equivalent to that observed after intramuscular injection of 90.5 $\pm$ 5.8% of this dose, but that this equivalent rapidly decreases on increase of the intake to amount only to 3.0 $\pm$ 0.7% at the dose of 50 μg B_{12}.

Fig. 2 illustrates the general principle of decreasing efficiency of absorption of vit. B_{12} in the intestine on increase of the dose ingested. The first curve in this figure which has a hyperbolic character, represents the regression of the efficiency of absorption of vit. B_{12} in the intestines with increase of the dose.

TABLE I. Intestinal Absorption of Vitamin B_{12} in Normals at Various Doses of Vitamin B_{12} Ingested.

Dose of radioactive vit. B_{12}, μg	No. of cases studied	Mean hepatic uptake with stand. error in counts/min. above background calculated per 1 microcurie of Co^{60} ingested*	% absorption as compared to parenteral = 100%	Parenteral equivalent of intestinal absorption, μg†
2-10 intramuscularly	4	885 ± 77	100.0 ± 8.7	
.5 orally	3	802 ± 51	90.5 ± 5.8	.45 ± .03
1.0 "	7	784 ± 110	81.5 ± 11.4	.81 ± .11
2.0 "	4	335 ± 72	40.0 ± 8.1	.80 ± .16
5.0 "	3	195 ± 29	22.0 ± 3.3	1.10 ± .16
20.0 "	3	53 ± 13	6.0 ± 1.5	1.20 ± .29
50.0 "	6	26 ± 6	3.0 ± .7	1.50 ± .35

* Each figure represents mean with stand. error averaged from increments in radioactivity uptake over 3-4 skin projections of the liver on several days between 6th and 10th day of experimental period, calculated per 1 microcurie of radioactive Co^{60} contained in dose ingested. Counts were taken with 1" x 1" sodium iodide thallium crystal at 1000 volts without collimator and amplifier, and in close contact with the skin.

† Since at the dose below 20 μg about 6% of vit. B_{12} injected is not retained in the body but excreted in the urine(20) real intestinal absorption figure is at least 6% less than parenteral equivalent listed in this column.

The second curve shows the increments in the absolute amounts of B_{12} absorbed on increase of the dose, which are strikingly small due to the regression of the efficiency of absorption on increase of the intake. Thus, the increment in the amount of vit. B_{12} ingested from 0.5 to 50.0 μg will result in an average increment of vit. B_{12} absorbed from about 0.45 to 1.5 μg, *i.e.*, only about 1 μg. The data have been computed on the basis of a limited number of determinations; individual differences in absorption may be considerable (Table I). It is obvious, however, that the efficiency of absorption of vit. B_{12} in the intestine is best in the physiological range of 0.5-1.0 μg.

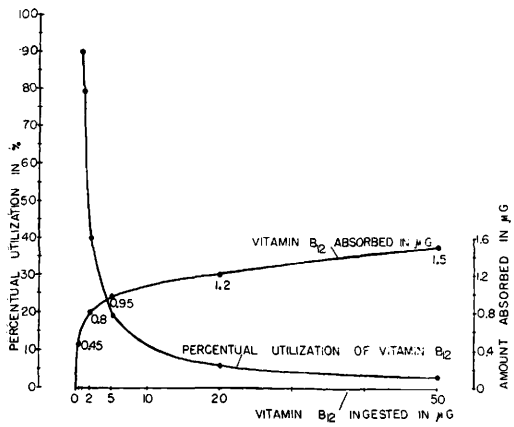

FIG. 2. Average intestinal utilization of vit. B_{12} in normal humans. Curves of the percentual utilization of vit. B_{12} and of the factual amounts of vit. B_{12} absorbed in the intestine were drawn on the basis of data listed in Table I, with exception of the figure corresponding to the dose of 5 μg vit. B_{12} ingested, which was calculated from earlier observations. Because of considerations mentioned in Table I, true values of absorption of vit. B_{12} in intestine are probably by about 6% less than those shown in this figure. Moreover, figures used here should be considered as mean values to which stand. errors listed in Table I should be applied.

Discussion. It appears that the intestinal absorption of vit. B_{12} is controlled by the existence of a partial mucosal block to its absorption which shows much similarity to that existing to the absorption of iron in intestine. There is evidence to show that the partial block to intestinal absorption of vit. B_{12} in normals is not related to the inadequate supply of gastric intrinsic factor of Castle(18,19), and that it cannot be removed in normals by addition of an excess of intrinsic factor containing material(18). This tends to indicate that under normal and pathological conditions it may require for absorption of vit. B_{12}, in addition to gastric intrinsic factor, also an intramural "intestinal B_{12}-acceptor" the role of which in B_{12} metabolism would be similar to that of apoferritin in iron absorption. After B_{12} passes through the mucosal membrane, a process for which the gastric intrinsic factor seems to be necessary(20), the vit. B_{12} would become bound to this B_{12}-acceptor. With in-

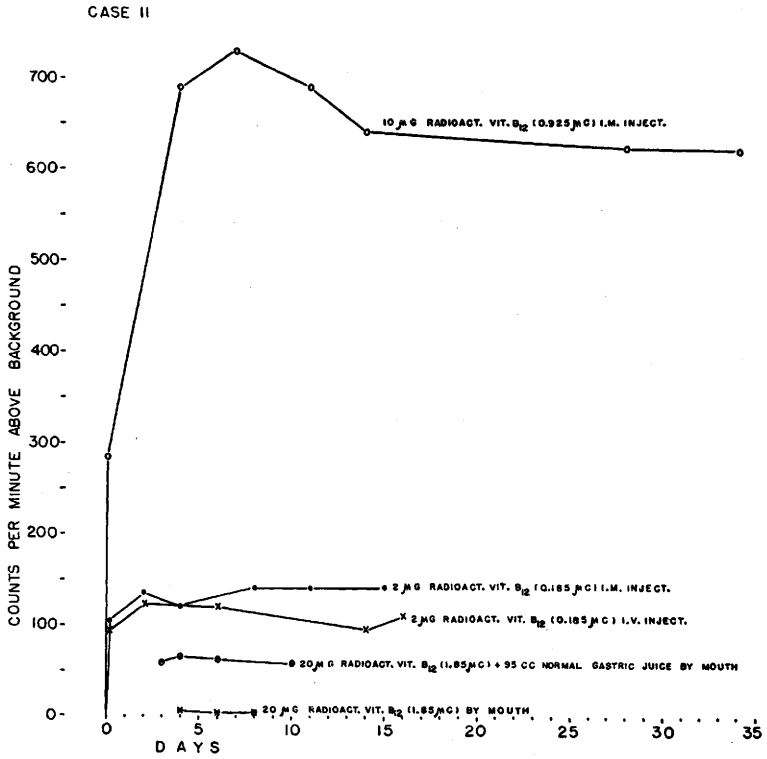

FIG. 3. Uptake of radioactive vit. B_{12} by liver in a patient with pernicious anemia in partial relapse following oral administration of this vitamin alone and with normal gastric juice, as well as after intramuscular and intravenous administration. The parenteral dose was 2 respectively 10 times smaller than the oral one which was 20 μg radioactive vit. B_{12} containing 1.85 microcuries of Co^{60}.

creasing saturation of the B_{12}-acceptor in the intestinal wall the absorption of vit. B_{12} in the intestine would be braked, which might explain the regression of efficiency of absorption of vit. B_{12} on increase of the dose. The gradual release from the intestine of B_{12} bound to this hypothetic intestinal B_{12} acceptor may also explain why it takes up to 7 days for the hepatic uptake of the orally administered dose of radioactive B_{12} to come to a peak(14-16). There is evidence that after intestinal absorption B_{12} becomes bound to one of the proteins in the serum(3,21,22), which, by analogy with the serum transferrin we might call "B_{12}-transferrin" and the function of which would consist in carrying the bound vit. B_{12} in blood. Ultimately, vit. B_{12} becomes anchored in the liver(14,15) and the hematopoietic tissues.

The partial mucosal block to intestinal absorption of vit. B_{12} changes to a complete or almost complete block in sprue and in pernicious anemia, as evidenced by no or a negligible hepatic uptake of orally administered vit. B_{12} in these diseases(14-16). In sprue, the block cannot be corrected by the addition of gastric intrinsic factor(14-16), because the defect in this disease depends on a generalized and inherent defect in the absorption mechanism of the intestinal wall. In pernicious anemia, the block to absorption of vit. B_{12} depends largely on the absence of Castle's gastric intrinsic factor and can be converted into a partial block, similar to that existing in normals, by addition to vit. B_{12} of normal human gastric juice(1,2,7,10), intrinsic factor concentrate from human or hog stomach (7-9), or by lavishly increasing the intake of vit. B_{12} alone, which through mass effect overcomes the block and results in absorption of some small fraction of the ingested dose(2,4). However, the principle of regressing efficiency

of absorption with increase of the intake still will hold under these circumstances (Fig. 3). In this patient with pernicious anemia the hepatic uptake of radioactivity was zero following the ingestion of an oral dose of 20 μg Co^{60}-B_{12} alone, but it became equivalent to about 5% of the similar parenteral dose when it was given together with a potent source of intrinsic factor. This is the usual range of efficiency of intestinal absorption of vit. B_{12} at the intake of 20 μg in normals (Table I).

The principle of regressing utilization of vit. B_{12} in the intestine permits a better understanding of difficulties encountered in the oral treatment of pernicious anemia with vit. B_{12}, and the apparent "unpredictability" of hematopoietic responses under these circumstances.

Summary. The scintillation measurements of the hepatic uptake of Co^{60}-B_{12} following its oral and parenteral administration to 20 normal humans, indicate that the efficiency of intestinal absorption of vit. B_{12} decreases sharply on increase of the intake. The peak of the absorption curve of vit. B_{12} was found at the oral dose of 0.5 μg, at which the hepatic uptake was found equivalent to 90.5 $\pm$ 5.8% of that observed following intramuscular injection of a similar dose of this vitamin. With the increase of the dose, a progressive decline in absorption followed a hyperbolic regression curve, so that at the oral dose of 50 μg B_{12} the hepatic uptake was equivalent to only 3.0 $\pm$ 0.7% of that found after intramuscular injection of a similar dose of this vitamin. The data obtained indicate that the increment in the oral dose of vit. B_{12} from 0.5 to 50.0 μg, results apparently in an increase of the amount absorbed of only 1.0 μg. It is suggested that in addition to Castle's gastric intrinsic factor, an intramural "intestinal B_{12}-acceptor" exists, which may be responsible for the partial mucosal block to the absorption of vit. B_{12} in the intestine of normal humans. The role of this hypothetic acceptor in the absorption of vit. B_{12} might be analogous to that of apoferritin in intestinal absorption of iron.

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