One U.S.P. unit is defined (*6*) as that amount of liver extract required daily to produce satisfactory clinical and hematological responses in addisonian pernicious anemia. If it is assumed that this new crystalline compound is the only substance present in these preparations which is therapeutically active,[6] it is evident that clinical response should be obtained from the parenteral administration of approximately 1 μg of the new vitamin/day. The clinical responses obtained with single 3- and 6-μg doses of crystalline vitamin B_{12} are not inconsistent with the approximate equivalence of 1 μg of the vitamin and 1 U.S.P. injectable unit. It should be pointed out, however, that it is customary to administer 20–60 U.S.P. units of liver extract during the first two or three days to start remission of pernicious anemia in relapse. This dose range is equivalent to not more than about 20–60 μg of vitamin B_{12}.

Further research is in progress on the composition, structure, and biological activity of vitamin B_{12}.

During this research, we have had the pleasure and benefit of the interest and advice of Henry D. Dakin.

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Activity of Vitamin B_{12} for the Growth of *Lactobacillus lactis*[1]

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A factor (LLD) required by *Lactobacillus lactis* Dorner was found (*3*) in refined liver extracts in concentrations bearing an almost linear relationship to the unit potency of the extracts used in the treatment of pernicious anemia. It was suggested that the LLD factor might be the therapeutically active principle in these extracts. A crystalline compound, vitamin B_{12}, has been isolated from liver (*1*) and has been shown to be highly active hematopoietically in initial tests upon cases of pernicious anemia (*4*).

Vitamin B_{12} has been assayed for LLD activity by essentially the method reported previously (*3*). The growth-promoting effect of crystalline vitamin B_{12} has been compared with that of a liver concentrate used as an arbitrary standard[2] for the LLD factor assay. The standard was assigned a potency of 1,000 units/mg. On this basis, the potency of vitamin B_{12} was found to be about 11,000,000 units/mg when a 23-hr growth period was employed and about 17,000,000 units/mg with a 42-hr growth period. These assay results illustrate the variation which may occur under varying test conditions. The conditions under which the culture is maintained may also affect the reproducibility of the assay values.

These results show that vitamin B_{12} is either wholly or partially responsible for the LLD growth activity observed for liver extracts. It is conceivable that two or more closely related principles may be present which are responsible for the activity observed. The multiple chemical nature of certain vitamins is now well known. The minute amount of this compound required for growth places vitamin B_{12} among the most potent microbiologically active compounds.

The liver concentrate standard is free from the other factor, TJ, required by *L. lactis*, at levels as high as 500 μg/tube. *L. casei, L. fermenti, L. arabinosus, Streptococcus faecalis* R, and *Escherichia coli* grow readily on the amino acid medium without supplementation with tomato juice. The addition of the liver concentrate, as a source of LLD factor, has little or no effect on their growth. The TJ factor(s) has been found to be synthesized by *E. coli, L. arabinosus, L. casei, L. fermenti*, and *Str. faecalis* R when they are grown on the amino acid medium.

Tests have been made for the presence of the LLD and TJ factor activities in those source materials and fractions derived therefrom which have been reported to contain unidentified factors for chicken nutrition (*2*). The LLD factor activity occurs in fairly high amounts, in approximately decreasing order, in a papain digest of acid precipitate from cow manure,[3] the acid precipitate from cow manure,[3] fish meal, pancreatin, papain, egg white, egg yolk, and in lower amounts in alcoholic extract of whey, potassium permanganate-oxidized alcoholic extract of whey, soybean oil meal, gelatin, zein and Mylase P. enzyme. The TJ factor activity is also found, in approximately decreasing order, in the papain digest of acid precipitate from cow manure, the acid precipitate of cow manure, egg yolk, papain, and pancreatin, but in much lower amounts in fish meal, alcoholic extract of whey, soybean oil meal, crude casein, egg white, zein, Mylase P. enzyme, potassium permanganate-oxidized alcoholic extract of whey, and gelatin. The distribution of

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[2] This liver concentrate standard and the crystalline vitamin B_{12} were supplied by Merck & Co., Inc., during collaborative studies.

[3] The acid precipitate and the papain digest of the acid precipitate from cow manure were kindly supplied by H. R. Bird, Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville, Maryland.

[6] The commercial liver preparations listed in the table were found to contain the equivalent of from 0.7 to 10.6 μg/ml of pteroylglutamic acid, as measured by chicken pancreas conjugase digestion followed by *L. casei* assay, using pteroylglutamic acid as the standard.

the LLD and TJ factor activities in these materials suggests that they may be involved in chicken nutrition.

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Activity of Vitamin B_{12} in Addisonian Pernicious Anemia

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Crystalline vitamin B_{12} has shown positive hematological activity in three cases of addisonian pernicious anemia.

Collaborative clinical studies were begun in 1942 with chemical investigators in the Merck Research Laboratories on the further purification of liver concentrates which are effective in the treatment of pernicious anemia. Recently, a correlation was found between clinical activity and the activity of concentrates for the growth of *Lactobacillus lactis* Dorner. Shorb (*3*) has reported a relationship of the unit potency of liver extracts used in the treatment of pernicious anemia to a factor (LLD) required by *L. lactis.* These researches led to the isolation from liver of a crystalline compound designated vitamin B_{12} (*2*), which is very potent for the growth of *L. lactis* (*2, 4*). Vitamin B_{12} has produced a positive hematological response in three patients following single intramuscular injections of 3, 6, and 150 μg, respectively.

Four patients receiving single injections of impure amorphous concentrates containing 20,000–40,000 LLD units gave strong or maximal hematological responses, while three receiving 10,000 LLD units or less gave weak or negative responses.

Results from the last case receiving amorphous material and the three receiving crystalline vitamin B_{12} are summarized in Tables 1 and 2. One mg of crystalline vitamin B_{12} is equivalent to about 11×10^6 LLD units; 1 μg, to about 11,000 units.

TABLE 1

Amorphous Liver Concentrates

Case	*P.H. 88–58–92*						
Day	0	6	14	23	29	42	119
RBC × 10^6	1.7	...	2.4	2.6	2.8	3.3	4.5
Hgb (gm)	6.8	...	7.8	9.5	9.8	11.0	14.0
Reticulocytes (%)	2.4	20.0	1.5	1.0	13.6	1.1	...
LLD units	2×10^4			1.8×10^6			

Case 88–58–92 went home on the 45th day and has received no treatment aside from that recorded. Case 9178–48 had eaten some liver before entering the hospital and had a reticulocyte count of 8% a week before day 0, by which time the count had fallen to 2.8%.

It is evident that there has been a rise in reticulocytes, red cell count, and hemoglobin, but it is still too early to say whether the blood picture will return to normal without further treatment in the last three cases. The rise in white count in case 9007 is striking; the platelets have risen from 120,000 to 340,000 in case 9178–48.

TABLE 2

Crystalline Vitamin B_{12}

Case	*Kings County 9007*				*Bellevue 8900–48*			*Bellevue 9178–48*			
Day	0	5	14	23	0	5	14	0	5	9	15
RBC × 10^6	1.5	...	2.6	3.4	1.5	...	2.6	1.4	...	...	2.6
Hgb (gm)	4.5	...	7.0	9.0	7.8	...	10.0	6.8	...	...	9.8
Reticulocytes (%)	0.5	27.0	2.0	0.5	2.8	26.0	3.1	2.8	10.2	4.0	2.6
Hct (%)	14.0	...	25.0	29.0	17.0	...	31	17	...	...	31
WBC × 10^3	9.0	25.0	9.0	...	2.3	...	5.8	4.2	...	...	8.1
Vitamin B_{12} (μg)	150	...	...	...	6	...	...	3	...	50	...
LLD units	1.6×10^6				6.6×10^4			3.3×10^4		5.5×10^5	

It is of interest that Preparation #31 of Dakin, Ungley, and West (*1*), which was clinically positive in 1936, has been shown by the microbiological assay to contain about 30,000 LLD units (3 μg B_{12})/25 mg, the dose used at that time.

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