VARIATIONS OF THE VITAMIN B_{12} CONTENTS OF VARIOUS TISSUES IN THE COURSE OF EMBRYONIC AND FETAL DEVELOPMENT

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It has been well established recently that vitamin B_{12} plays a vital role in the nutrition of man, other animals and certain micro-organisms. Actually, vitamin B_{12} is considered to be implicated in some of the biochemical reactions, such as methyl group synthesis, desoxyriboside synthesis, activation of sulfhydryl enzymes and protein synthesis. [1–3]. It is thus one of the essential growth factor in human nutrition [4] and in the maintenance of normal health, [5] in addition to its antipernicious anemia activity.

However, the bacteria is known to be the chief source of vitamin B_{12}, whereas higher plants and animals are generally unable to synthesize it. The works of Chun [6] and others [7–9] on the maternal and fetal serum at parturition suggest, however, that certain animal tissues may have this ability; and activities of vitamin B_{12} in tissues of different origin during embryonic and fetal development would pose an interesting problem.

It appears therefore that, by analogy, conditions might be favorable for the synthesis of vitamin B_{12} in the developing chick embryo. And it must be noted that embryonic and fetal development implies a problem of cellular and tissue synthesis and the need for facilitation of all mechanisms involved in such synthesis is pressing. Vitamin B_{12} is known to be one of the vitamins which are involved in this phenomenon [10].

Because the pregnancy forces its abnormal demands on maternal metabolism, it would be quite logic to consider that the available stores of the vitamin are brought into circulation to continue this state and to further fetal development. But there has been a minimum of data to elucidate the whole pictures involved, though numerous studies on the metabolic role of vitamin B_{12} have been reported.

In the present study, an experiment was designed to follow up the viariation of the vitamin B_{12} contents in the various organs of embryo and fetus through-out their developmental courses.

EXPERIMENTAL

MATERIALS

Experimental animals used in the present investigation comprise as follows, white leghorn eggs, human fetus, pigs and rabbits.

The eggs were from the white leghorn hens fed on a mixed diet contaning animal protein. They were incubated in a forced-draught incubator at 100°F and with suitable relative humidity, turning their position three times a day until the fifth day from beginning and twice, thereafter. The eggs were examined on the fifth day of incubation whether they were fertilized, excluding unfertilized, and dead ones.

The incubated eggs were then frozen at −50°C after 12, 14, 16, 18 and 20 days of incubation periods for making no further development and for complete comminution of all tissues. The 12-day incubated group consisted of 10 eggs, 14-day of 8, 16-day of 6, 18-day of 5, and 20-day of 8 eggs respectively.

Human fetuses were obtained from the abortive cases of pregnant women hospitalized at the Obstetrical ward, University Hospital, Medical school, Chonnam University. They are 10 cases in total; one 5-month, 6-month, four 8-month and three 10-month abortive and still birth cases. Aborted fetus of each case were immediately subjected to operation to their livers and kidneys, which were kept frozen for further sample preparation and analysis.

Pregnant pig of unknown species, the body-weight of which was 168 kg. at the time of expected day, was purchased for the present investigation. On the expected day after pregnant period (normal: 105 days), the pregnant pig was killed, and 13 fetal pigs were obtained by immediate operation. Various tissues from mother pig and fetal pigs were kept frozen immediately as did in other cases for analysis.

Two pregnant rabbits of unknown species were also purchased and sacrificed on their expected day (normal 30 days) and their further treatment was the same as described for the pregnant pig. However, the tissues of the fetal rabbits was unfortunately spoiled by an accident, for which the author feels greatly discouraged.

METHOD

Preparation of tissue homogenates: The determination of B_{12} in organs was made on tissue homogenates prepared with carefully measured amounts of distilled water, either in a Waring Blender or the Potter-Elvehjem homogenizer. Unless examined immediately, the homogenates were kept frozen. For preparing these homogenates, the shells of incubated eggs of various stages were removed, followed by separation of liver, heart and lung. Since the small quantities of every organ in a chick embryo permit no separate analysis, same tissues of each group were pooled together to eliminate possible errors to be encountered. Therefore, the values obtained by triplicated analysis on these pooled samples were given with their mean values in the

results.

Tissues from human fetus, pigs and rabbits were blotted on the filter paper after perfusing with distilled water, and the tissues of same group were all pooled for triplicated analysis, let alone the tissues of chick embryo in order to avoid bio-statistical errors.

Microbiological assay: Vitamin B_{12} was determined microbiologically by the *Lactobacillus leichmanni* assay originally described by Skeggs et al. [11] The reasons for the choice of this method have been appeared elsewhere [7], but additional comment may be in order. Both *Lactobacillus leichmannii* and *Euglena gracilis* have been widely used as the test organisms for vitamin B_{12} assay, and both have been criticized on the basis of their growth responses to factors other than vitamin B_{12}, notably thymidine and desoxyribosides. Recently *Ochromonas malhamensis* has been reported to be free of this criticism [12] and the results obtained with it may be a true measure of vitamin B_{12}. But this method requires longer culture with frequent shaking. Though differential responses of the various organisms are known, the amount of thymidine and desoxyribosides in the samples were less significant. Accordingly, the *Lactobacillus leichmannii* method was chosen for present investigation.

The stock cultures were prepared with a tomato juice yeast extract agar (AOAC, 1955) [13], in which the test organism, *Lactobacillus leichmannii*, was cultured; and for the broth subcultures of the organism the same medium except the agar was used. The assay medium of the organism was prepared after the commercial vitamin B_{12} assay broth described in the U. S. P., modified in 1951 [14].

In order to prepared the inoculum for assays, the 24-hour subcultures of the test organism were subjected to centrifugation, after which the residue was washed three times with the normal saline. Adjusting the turbidity of the suspension of this cells to 70% light transmission, one drop was introduced by a 10-ml. syringe into each assay tube at the time of inoculation. The assay tubes utilized were 16×150 mm. in size and graduated with careful calibration.

One ml. of tissue homogenates was added to 10 ml. of acetate buffer, pH 4.5, prepared by mixing equal volume of 0.1 *N* acetic acid and 0.1 *N* sodium acetate, and autoclaved at 100°C. for 30 minutes in the presence of a trace amount of potassium cyanice. Portions of the extracts were clarified by centrifugation, neutralized with 0.15 *M* potassium phosphate and brought to appropriate volume by diluting 20 times. The diluted aliquots were used for each triplicated assay.

As the standard, the vitamin B_{12} solution (50r/ml.) purchased from the American Pacific Laboratory was used, diluting it appropriately to bring the working concentration 0.02mr/ml. Together with a series of this standard solution of varying concentrations, samples and blanks were set up in triplicate, one not being inoculated and two others being inocluated. All tubes of assay series (standard, samples and blanks) made up to a definite volume with distilled, sterilized wathe were cotton-plugged and autoclaved for 5 minutes under the pressure of 15 pounds, allow to

stand a while for cooling, inoculated and finally incubated for 24 hours at 37°C.

Responded growths of the test organism were determined turbidimetrically using the Coleman spectrophotometer at 525 mu, setting uninoculated blank at 100% transmission, and expressed as vitamin B_{12} activities.

RESULTS

As already mentioned, standard determinations of varying concentrations were parallelly performed with each series of sample assay to avoid possible fluctuations of the standard curve. Several series of the standard curves *per se* for each assay were in good accord with each other and with that obtained by Berman et al. [15]

All the assay data did not vary by more than ±5% as compared to respective mean values as they appear in the Table 1 through Table IV.

Table 1 shows the triplicated analytical values with their means for vitamin B_{12} in the liver, heart and lung of the eggs at 12, 14, 16, 18 and 20 days' incubation. Throughout the course of 20 days' incubation, Table 1 reveals a substantial increase during the development of the embryo; liver from 88 mγ/gm. to 192.2 mγ/gm., heart from 52.0 mγ/gm. to 86.2 mγ/gm., and lung 78.8 mγ/gm. to 140.0 mγ/gm. And, in general, it may be clearly noted that the vitamin B_{12} contents per gm. tissue is the highest in the liver, second in the lung and the lowest in the heart in any group incubated, through the pattern of gradual increase during embryonic development shapes up some appreciable differences among them (Fig. 1).

The Vitamin B_{12} content in the heart of 12 days incubated chick embryos shows 60% increase at the end of more successive incubation of 8 days, while that in the

Table 1 Triplicated analytical values and their means of the vitamin B_{12} contents (mγ/gm.) of developing hen's eggs.

Materials	Incubation pirouds 12-days (10)*	14-days (8)*	16-days (6)*	18-days (5)*	20-days (8)*
Liver	89.4	117.4	121.5	138.6	190.0
	85.5	122.7	118.0	143.0	194.5
	89.0	122.0	122.5	138.5	192.0
	88.0	120.0	120.3	140.0	192.2
Heart	50.0	73.0	75.8	85.0	84.5
	53.2	75.0	77.5	79.8	88.0
	52.9	74.5	77.0	80.0	87.0
	52.0	74.5	76.8	81.6	86.2
Lung		77.8	110.0	121.8	139.8
		79.5	109.0	118.0	141.0
		79.0	105.0	120.3	139.1
		78.8	108.0	120.0	140.0

* The numbes of individual hen's egg is shown in parenthesis.

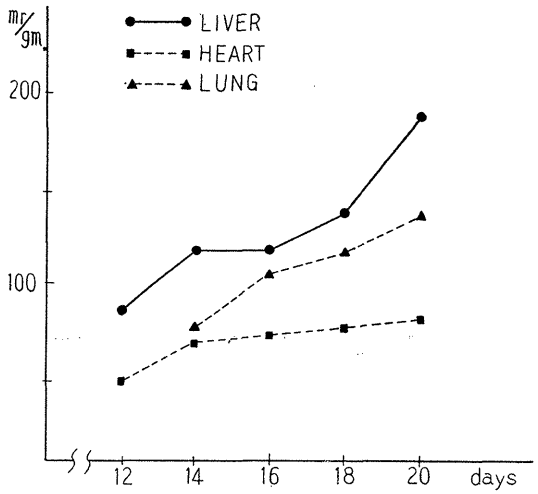

Fig. 1 Variations of the vitamin B_{12} contents in liver, lung and heart of developing chick embryo.

lung does approximately 80% and that in the liver does almost as much as 120%.

Table II shows the results of same observation made on the human fetus during development. In the human fetus as shown in Table II and Fig. 2, the trend of variations in the course of fetal development is exactly comparable to that observed in the chick embryos.

Liver contents of vitamin B_{12} from 5-month through 10-month old fetus are 135.5 mr/gm. to 170.6 mr/gm. while kidney contents are 128.0 mr./gm. to 150.1 mr/gm. with an exception of slight decrease in 6-month old kidneys. However, the increment in the quantities of the vitamin B_{12} in both organs is not so much clear-cut as observed in the developing chick embryo.

Table II Triplncated analytical values and their means of vitamin B_{12} contents (mγ/gm.) of the human fetus during development.

Materials	Intra-uterine periods			
	5-month (1)*	6-month (2)*	8-month (4)*	10-month (3)*
Liver	130.0	155.0	168.0	176.7
	130.0	151.0	160.0	168.0
	137.6	160.0	152.0	167.2
	135.5	155.3	160.0	170.6
kidney	118.5	124.0	136.0	152.0
	148.0	119.5	139.5	148.0
	116.5	118.0	132.5	150.4
	128.0	120.5	136.0	150.1

* The number of fetus in each aborcive case is indicated in parenthesis.

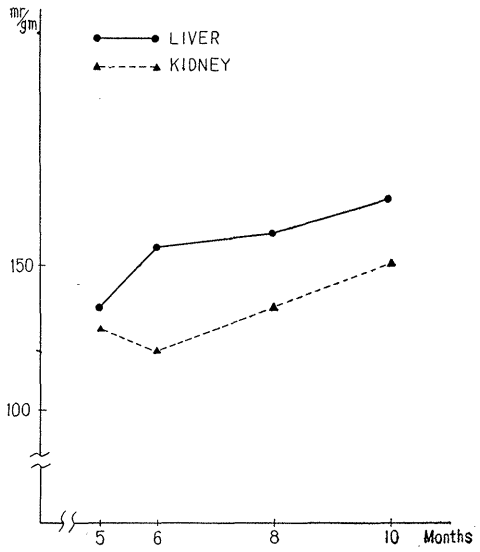

Fig. 2 Variations of the vitamin B_{12} contents in liver and kidney of developing human fetus.

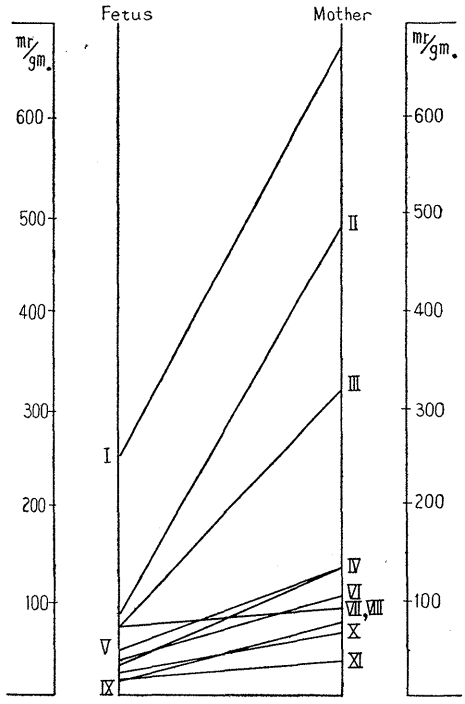

Fig. 3 Comparison of the vitamin B_{12} contents in the various organs of maternal and fetal pigs.
I. Pancreas II. Liver III. Lung IV. Large intestine V. Small intestine VI. Brain VII. Kidney VIII. Stomach IX. Tongue X. Heart XI. Muscle

Table III Comparison of triplicated analytical and mean values of the vitamin B_{12} contents in various tissues of maternal pig and fetal pigs. (mγ/gm.)

Material	Fetus (13)*	Mother (1)*	Material	Fetus (13)*	Mother (1)*
	252.5	672.6		69.0	81.8
	247.5	679.0		69.0	79.0
Pancreas	250.0	688.5	Stomach	72.0	79.3
	250.0	680.0		70.0	80.0
	100.0	495.0		18.4	72.4
	86.9	495.7		15.0	64.0
Liver	102.0	497.3	Tongue	17.0	65.2
	96.3	496.0		16.8	67.2
		336.5		29.0	64.5
		335.2		29.0	63.0
Spleen		336.3	Heart	26.0	70.0
		336.3		28.0	65.8
	70.0	322.1			36.7
	69.0	322.0			37.5
Lung	70.8	328.0	Esophagus	—	37.5
	70.0	324.0			37.2
		142.6		19.0	35.0
		143.2		18.8	34.0
Placenta	—	140.0	Muscle	19.8	38.0
		145.6		19.2	35.6
	38.5	141.2			25.8
	35.6	135.7			27.0
Large intestine	35.0	136.0	Bladder	—	32.0
	36.4	137.6			28.3
	49.5	143.3			24.0
	49.5	139.5			24.0
Small intestine	49.8	130.0	Ureter	—	24.0
	49.6	137.6			24.0
	43.5	114.0		5.4	
	42.5	108.0		5.0	
Brain	40.0	107.6	Cord	6.3	—
	42.0	109.9		5.6	
	68.8	80.0			
	70.0	77.7			
Kidney	70.5	82.3			
	70.1	80.0			

* The number in parentesis indicate either the number of maternal pig and fetal pigs, the same tissue of which are pooled.

It may well be said that the difference of vitamin B_{12} increament between the chick embryo and human fetus could possibly ascribable to the different growth rate.

It is, however, noted that there is no appreciable difference between them with regard to their liver contents of vitamin B_{12}.

In the Table III, the results of vitamin B_{12} assay on various organs of mother pig and her 13 fetus, are presented for comparison. Fig. 3 presents the paired values of tissue vitamin B_{12} for mother pig and fetus. As one can notice apparently in Table III and Fig. 3, the vitamin B_{12} contents of the maternal tissues is consistently and considerably higher than that of fetal tissues. Most drastic differences between maternal and fetal contents of vitamin B_{12} are those observed in liver, lung and tongue, the ratio of maternal to the fetal contents being 5.1, 4.6 and 4.0 respectively.

Whatever the difference between them may be, the contents of vitamin B_{12} in various organs are the highest in pancrease, liver, spleen, lung, placenta, large intestine, small intestine, brain, kidney, stomach, tongue, heart, esophagus, muscle, bladder, ureter, and cord in the decreasing order in both maternal and fetal tissues, with the exceptions of fetal kidney and stomach (Fig. 3). Among fetal tissues, 250.0 mγ/gm. for pancreas is strikingly higher than all the contents of the rest tissues; and among maternal tissues, 680.0 mr/gm. for pancreas, 496.0 mr/gm. for liver, 336.3 mr./gm. for spleen and 324.0 mr./gm. for lung are also remarkably higher than others.

Table IV presents the vitamin B_{12} contents of various tissues of two pregnant rabbits. The mean vitamin B_{12} contents of the various tissues in decreasing order are as follows; kidney, liver, heart, uterus, pancreas, ovary, stomach, lung, large in

Table IV Triplicated assay data and their mean values of the vitamin B_{12} contents (mr/gm.) in various organs of the pregnant rabbit.

Materials	Triplicated Values	Means	Materials	Triplicated Values	Means
Kidney	587.0 612.0 601.0	600.0	Stomach	158.5 164.0 157.5	160.0
Liver	370.0 389.8 362.7	374.2	Lung	94.5 92.0 94.0	93.5
Heart	360.0 367.0 352.5	360.0	Large intestine	93.5 95.0 92.0	93.5
Uterus	235.0 218.5 222.0	225.2	Brain	89.0 86.5 87.0	
Pancreas	219.0 215.5 225.5	220.0	Small intestine	73.5 75.5 76.0	75.0
Ovary	188.0 179.5 190.0	185.8	Muscle	67.5 65.5 66.5	66.5

estine, brain, small intestine and muscle. Only kidney, unlike that of pigs, has the remarkably higher content of vitamin B_{12} as much as 600.0 mr/gm. than do the rest tissues.

DISCUSSION

As to the variations of the vitamin B_{12} contents of incubated eggs, Fischer et al. (16), using the *Lactobacillus leichmannii* as the test organism, found that the mean values per egg increased from 0.12 μg/at 0 days to a maximum of 0.4 μg. at 14 days' and then decreased to 0.12 μg. at 20 days' incubation. Skarz'ynski et al. [17] also reported that the vitamin B_{12} activities per egg for *Euglena gracilis* increased progressively during incubation (0.88 μg. to 3.62 μg. per egg), while the activities for *Ochromonas malhanmensis* showed no sign of increase.

The increasing trend of mean values obtained by Fischer et al. is essentially the same as the author's results quoted in Table I, though their determinations were done on the whole embryonated egg. Skarz'ynski et al [17] explained, pointing out that Fischer's value are considerably lower than theirs, that the lowered values of the latter are resulted because cyanide was not used when preparing the samples for assay. They have gone so far as to say that, if the low values, as seems likely, found by Fischer et al were due to incomplete extraction of the vitamin from their egg samples, their conclusion about changes in the vitamin B_{12} contents of embryonated eggs during incubation are open to doubt. So far as the cyanide adding is concerned, Denton & Kellogg [18] have already shown that the value for the vitamin B_{12} contents is several times higher when cyanide is used. However, increasing trends of their data *per se*, regardless the absolute activities of vitamin B_{12} was in good agreement with the author's value (Fig. 1). But Coats and Porter [19] suggested, referring the Fischer's higher values observed at the later stages of incubation as mentioned above that they may have been caused by unspecific growth factors, for which they did not show any reasonable evidences. But in the author's opinion, it is widely open to question to discuss the increase of the vitamin B_{12} activities during incubation on the whole egg alone, since the activities in the yolk are decreasing as the incubation proceeds as observed by Fischer et al [16] and Skarz'ynski et al [17] themselves. Therefore, it might be more logic to postulate that the vitamiin B_{12} would presumably transferred to individual developing tissues from the stored vitamin B_{12} in yolk fraction. The vitamin B_{12} in the yolk was found actually to be greatly reduced by 12 or 14 days of incubation, associated with a decrease in the total amount of yolk. [16] By the 12th day significant amounts of the vitamin are present in the yolk sac and 50% of the total vitamin in the egg has been transferred to the embryo.

The same assumption is said to be true of the cases observed in the human fetus during their intrauterine life presented in Table II and Fig. 2. That is, those trends of increase in the vitamin B_{12} activities appeared both in chick embryo and in hu-

man fetus could possibly ascribable to the suggested fact that vitamin B_{12} stored in the maternal tisses is transferred to fetal tissues through the placenta and that those stored in yolk fraction to individual embrycnic tissues through blood circulation.

This unique property of the fetus to concentrate vitamin B_{12} reported previously [8, 9, 20-25]. Chow [26] reported on account of this trend; where radioactive vitamin B_{12} was injected into pregnant rats about 2 weeks before parturition, and in relation to the body weight, a large portion of the injected radioactivity, taken as a measure of vitamin B_{12}, was found in the fetus. Choi and 0 [6] recently confirmed this on 40 parturient mother and their fetus analyzing the vitamin B_{12} contents in the serum. Boger et al. [9] also reported their study on 96 paired serum samples from mothers and infants (umbilical cord blood) and demonstrated that the average serum vitamin B_{12} concentration of the infant is approximately twice as that of the mother.

As can be seen in Fig. 2 in the present investigation, until the full term intrauterine life, liver and kidney of the human fetus concentrate the vitamin B_{12} progressively as the fetal development proceeds. There are still others besides vitamin B_{12}, known to be concentrated in the offspring in a manner similar to vitamin B_{12}, namely ascorbic acid [27], folic acid [8] and pyridoxine [28].

Quite recently, Helleger and Okuda [29] demonstrated an increased absorption of orally administered vitamin B_{12} in pregnant human and pregnant rat as compared to a series of non-pregnant controls. The inference was made that the fetus is the main beneficiary of the increased absorption. This assumption was primarily based upon the above-mentioned observations and those obtained in rat experiments which showed not only an increased vitamin B_{12} absorption in pregnancy, but also a reduced activities of vitamin B_{12} in maternal target organs (liver and kidney). Therefore, it was considered that such findings were best explained by the hypothesis of selective placental activity whereby fetal demand for the vitamin could be satisfied. By the previous confirmation done on the serum vitamin B_{12} activities by Choi and 0 [6] and the author's present results, it is apparent that pregnancy set forth an abnormal demands on the maternal pattern of metabolism and the fetus has to grow on excess nutrients as it is the main beneficiary in the body of mother.

As pregnancy exacts an overwhelming demand on the vitamin B_{12} store and presumably thereafter the fetus draws the vitamin from the maternal circulation, the author think confirmed that each tissue of the developing chick embryo draws vitamin B_{12} from the stored contents in the yolk as incubation takes place and vascularization throughout the yolk sac develops widely and diffusely; the more it draws the more it develops.

For futher confirmation for this assumption, another evidence reported by Wagle et al [3] is very much suggestive; uniformly lobelled glucose and beta-labelled serine were administered to young vitamin B_{12} deficient rats and pigt, and the incorporation of C^{14} was followed in TCA precipitated liver protein. Precipitates obtained from the vitamin B_{12} deficient animals were found to contain less radioactivity than

similar preparations obtained from the adequately vitamin-B_{12} supplemented animals. This means, of course, vitamin B_{12} is one of the factors to accomodate protein biosynthesis, which casts a clue for understanding of fetal increase in the content of vitamin B_{12} in the developing fetus and chick embryo.

Moreover, since a dominant factor in embryogenesis of the chick appears to be a high rate of cell division and accumulation of DNA [30], and the growth of the vitamin B_{12} deficient embryo as measured by dry weight or total nitroten is significantly retarded at 18 and 21 days of age, [31] the observed results of the present investigation as to the increase of the vitamin B_{12} contents in various embryonic and fetal tissues during their development are thought to be direct consequence resulted from developmental needs.

As it is apparent, on the other hand, from Table III and Fig. 3 that the absolute contents of vitamin B_{12} in various tissues of pregnant pigs just before delivery were, generally much higher than those of its fetus; the ratio of maternal over fetal contents being 5.1 the highest in liver, 4.6 in lung, 4.0 in tongue, 3.7 in large intestine, 2.7 in both pancreas and small intestine, 2.6 in brain, 2.3 in heart, 1.8 in muscle, and 1.1 in both kidney and stomach. As Fig. 3 reveals, particularly higher contents on the maternal side were observed in pancreas, liver and spleen, which also exhibit the higher contents in fetal side; and this parallelism appeared consistent in every tissue compared, except for the kidney and stomach.

Comparing the contents of vitamin B_{12} in various tissues of pregnant pig and rabbits (Table III and IV), discrepances in the contents and their relative order of vitamin B_{12} activities are noted. In the pregnant rabbits, kidney shows the highest value, almost as much as two fold of the other tissues. Though numerous studies so far presented with regard to the vitamin B_{12} contents of various tissues of domestic animals, none of them appeared to be in good identity in their quantities with each other. The vitamin B_{12} activities of rabbit liver, kidney and spleen, however, have been reported as comparable to those of chick [32, 33] but the similarity is not substantiated in more recent reports [34]. Liver and kidney tissues from rabbit, cattle and sheep also appear to contain similar concentrations of the vitamin B_{12} [35].

On the other hand, beef liver may contain 10 to 20 times more vitamin B_{12} activity than rat liver [63]. Differences of vitamin B_{12} activities in the tissues from different animals may be attributed to species differences [37], but a comparison of reports for tissues of the same species indicates a wide variation which has not been adequately studied. For example, Pitney et al [38] report 0.28 μg vitamin B_{12}/gm. while Swenseid et al. [39] report an average of 0.70 μg/gm for the human liver while Cooperman et al [40] reported 0.072 μg/gm. for dog's liver. They also presented varying vitamin B_{12} concentrations of pituitaries of different species; 0.491 μg/gm. for rabbit's, 0.42 μg/gm. for dog's, 0.27 for rat's and 0.203 ug/gm. for human infant.

Moreover, dietary intake is, in part, reflected in the concentration of vitamin B_{12} in tissues [41]. Therefore, it is almost impossible to compare the vitamin B_{12} activities

of different species of animals. Paticularly it is uncomparable when the animals are pregnant as is apparent in the present investigation.

However, the vitamin B_{12} contents of all tissues in maternal pig are distinctly higher than the contents of its offsprings' tissues. As far as the following organs concerned, kidney, liver, heart, pancreas, stomach, lung, large and small intestine, brain and and muscle; it is apparent that kidney, heart and stomach contents of vitamin B_{12} appeared to be higher in pregnant rabbit than those of pregnant pig, and that the rest of the organs listed above are higher in pregnant pig than in pregnant rabbit in their vitamin B_{12} activities.

Therefore, it would be of interest to determine whether the amount of vitamin B_{12} concentration of a tissue is related to the mitochondrial density of the cell where the vitamin B_{12} is located or to the particular metabolic rate or function of the tissue.

SUMMARY

By the microbiological assay, utilizing *Lactobacillus leichmannii* as a test organism, variations of the vitamin B_{12} contents of various tissues in the course of development of chick embryo and the human fetus are observed. A comparison between the vitamin B_{12} contents in various tissues of maternal pig and its fetus, along with an observation on the vitamin B_{12} contents in various tissues of pregnant rabbits are also made.

(1) The vitamin B_{12} contents in liver, heart and lung of the chick embryo increased progressively from 12 days' to 20 days' incubation as the embryonic development proceeds; liver contents being the highest, lung the second and heart the lowest throughout the incubation periods.

(2) The vitamin B_{12} contents in liver and kidney of the human fetus increase gradually from five-month through ten-month intra-uterine life; liver contents being higher than kidney contents throughout the developing periods.

(3) The vitamin B_{12} contents in various tissues of maternal pig are all higher than those of its fetus.

(4) The vitamin B_{12} contents in various tissue of pregnant rabbit are determined.

(5) Probable reasons for the increase in the vitamin B_{12} contents of the various tissues of developing chick embryo and human fetus are discussed.

REFERENCES

1) Schweighert, B. S., Nayder, O. F., Batzer, and Siedler, A. J.; *Radioisotopes in Scientific Research*, Pergamon Press, p. 297 (1959)
2) Wagle, S. R., David, A. V, Mistry, S. P., and Johnson, B. C.; *J. Biol. Chem.*, **230**, 2 (1958).
3) Wagle, S. R., Ranjam M., and Johnson, B. C.; *ibid.*, **230**, 1 (1958).
4) Wikes, F., Badenoch, J., and Sinclair, H. M.; *Amer. J. Clin. Nutr.*, **3**, 375 (1955).
5) Jolleffe, N., Funaro, R., Frontali, G., Maggioni, G., Garbo, S., and Landiano, G.; *Nutrition*

Symposium, Series No. 7, The National vitamin Foundation, New York (1953), p. 119.
6) Chun, H. O.: *Korean J. Med.*, **2**, 6 (1959).
7) Choi, Y. S. & Oh, S. K.: *Nippon Idai Zassi* **27**, 124 (1960)
8) Baker,H., Ziffer, H., Pasher, I, and Sobotka, H.: *Brit. Med. J.*, **1**, 978 (1958).
9) Boger, W. P., Bayne, G. M., Wright, L. D., and Beck, G. D.; *New Eng. J. Med.*, **256**, 23 (1957).
10) Jukes, T. H., Groquist, H. P., and Stokstad, E. L. R.; *Arch. Biochem. & Biophys.*, **26**, 157 (1950).
11) Skeggs, H. R., Nepple, H. N., Valentik, K. A., Huff, J. W., and Wright, L. D.; *J. Biol. Chem.*, **184**, 211 (1950).
12) Baker, H., Sobotka, H., Pasher, L., and Hutner, S. H.; *Proc. Soc. Exper. Biol. & Med.*, **91**, 636 (1956).
13) Association of Official Agricultural Chemists; *J. Assoc. Off. Agr. Chem.*, **30**, 846 (1953).
14) *Pharmacopoeia of the United States*, The 3rd Supplement, The 14th Revision, (1951), p. 15.
15) Berman, D., Yacowitz, H., and Weiser, H. H.; *Applied Microbiol.*, **4**, 49 (1956).
16) Fischer, N. A., Banson, E. M., and Swendseid, M. E.; *Arch. Biochem. & Biophys.*, **74**, 2 (1958).
17) Skarzynski, B., Zak, A., Ostrowski, W., Davis, M. K., Gregory, M. E., and Kon, S. K.; *Brit. J. Nutr.*, **13**, 72 (1959).
18) Denton, C. A., and Kellogg, W. L.; *Arch. Biochem. & Biophys.*, **46**, 105 (1953).
19) Coates, M. E., and Porter, J. W. G.; *Ann. Rev. Biochem.*, **28** (1959), p. 439.
20) Killander, A., and Vahlqist, B.; *Nord. Med.*, **51**, 777 (1954).
21) Molling, D. L., and Ross, G. I. M.; *J. Clin. Pathol.*, **5**, 129 (1952).
22) Nieweg, H. O., Faber, J. G., De Vries, J. A., and Kroese, W. S.; *J. Lab. Clin. Med.*, **44**, 118 (1954).
23) Okuda, K., Helleger, A. E., and Chow, B. F.; *Amer. J. Clin. Nutr.*, **4**, 4 (1956).
24) Boger, W. P., Wright, L. D., Beck, G. D., and Bayne, G. M; *Proc. Soc. Exp. Biol. & Med.*, **92**, 140 (1956).
25) Baker, H., Erdber, R., Pasher, I., and Sobotka, H.; *ibid.*, **94**, 513 (1957).
26) Chow, B. F., Barrow, L., and Ling, C. T.; *Arch. Biochem. & Biophys.*, **34**, 151 (1951).
27) Javer, C. T., and Standes, J.; *Surg. Gyn. & Obst.*, **76**, 115 (1943).
28) Glendening, M. B., Cohn, A. M., Page, E. Q.; *Proc. Soc. Exp. Biol. & Med.*, **90**, 25 (1955).
29) Helleger, A. E., Okuda, K., and Chow, B. F.; *Amer. J. Clin. Nutr.*, **5**, 327 (1957).
30) Solomon, J. B.; *Biochem. et Biophys. Acta*, **24**, 3 (1957).
31) Yesair, D. W., McOsker, D. E., and Daniel, L. J.; *Arch. Biochem. & Biophys.*, **79**, 168 (1959).
32) Yacowitz, C. H. L., Norris, C. and Hauser, G. F.; *Proc. Soc. Exp. Biol. Med.*, **79**, 279 (1952).
33) Kulwich, R., Struglia, L., and Pearson, P. B.; *J. Nutr.*, **49**, 639 (1953).
34) Shenoy, k. G., and Ramasarma, G. B.; *Arch. Biochem. & Biophys.*, **51**, 371 (1954).
35) Moinuddin, M., and Bentley, O. G.; *J. Nutr.*, **56**, 335 (1955).
36) Lewis, U. J., Register, U. D., and Elvehjem, C. A.; *Proc. Soc. Exp. Biol. Med.*, **71**, 509 (1949).
37) Miller, A., Gaull, G., Ross, J. F., and Lemon, H. M.; *ibid.*, **93**, 33 (1956).
38) Pitney, W. R., Beard, M. F., and Van Loon, E. J.; *J. Biol. Chem.*, **212**, 117 (1955).
39) Swendseid, M. E., Hvolboll, E., Schick, G., and Halsted, J. A.; *Blood*, **12**, 24 (1957).
40) Cooperman, J. M., Luhby, A. L., Teller, D. N., and Marley. J. F.; *J. Biol. Chem.*, **235**, 1 (1960).
41) Sheid, H. E., Andrews, M. M., and Schweigert, B. S.; *Proc. Soc. Exp. Biol. & Med.*, **78**, 558 (1951).