STUDY ON THE FACTOR IN BLOOD AND TISSUE WHICH PROMOTES YEAST GROWTH

Munenori YAMAKAWA

Department of Physiology,
Faculty of Veterinary Medicine,
Hokkaido University, Sapporo, Japan.

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As substances promoting yeast growth, there are available: zinc, manganese and copper which are inorganic substances, and some vitamins, some kinds of amino acid and ACTH, a hormone, all of which are organic substances. Besides, some other substances of this kind have been reported that increase or decrease in accordance with the living body conditions. The function of these substances have been discussed. This is a matter of great interest to physiology or to pathological physiology.

FILATOV reported that when a living body or its survived tissue was put under an unfavourable condition, some substance was organized in it against that condition. This substance was named biogenitic stimulant because of its characteristics as a stimulator of body functions and of yeast fermentation. The chemical composition was described to be a complicated mixture including some kinds of organic acids, although it has not yet been studied sufficiently. ISOGAWA found a promoting factor in refrigerated human blood and placenta. The factor was reported as the same or similar to FILATOV's biogenitic stimulant and as a kind of polypeptide from the chemical view point. HONMA et al. found a growth promoting factor in the sera of a normal horse and of cases of equine infectious anemia. This substance was considered to be, chemically speaking, between dipeptide and mucoprotein and to take in a living body the same part as a growth promoting factor, a kind of MENKIN factor. Further, KAMEYAMA found a promoting factor existing in the serum of men suffering from various diseases, in the morbid tissues of man, of horse, and of pig, and also existing in the serum of rabbit vaccinated. This factor was believed to differ from a biogenitic stimulant and to be a kind of protein hydrolyzate which has some relation both to carbohydrate metabolism and to the repairing of the living body.

These fore-going reports were made published about the growth promoting factor of yeast and about the physiological functions of these above noted substances in the body of animals. But, unfortunately, all the results were derived from unrefined materials for experiment and consequently it may be suspected that

many substances concerned with yeast growth can be contained in these reported substances. From this point of view, the functions of these promoters may be diverse in the body, and so the physiolosical meanings of these substances should be deliberately studied. It is necessary, undoubtedly, to attempt to refine these substances, but it is significant as well to investigate the increase and decrease of these substances, corresponding to physiological phenomena and also their distribution in the body.

In this papper, are reported the results of investigations undertaken from this standpoint.

Materials and Methods

As materials, use was made of blood and tissue of horses, of cows, of pigs and of sheep which were killed at Sapporo slaughter house, while blood of rabbits and of tuberculous patients was used occasionally. Tissues were homogenized by the homogenizor and then from a certain amount of the homogenate and of whole blood a growth promoting substance was extracted with double quantity of 95% ethanol for 24 to 48 hours. The extracted solutions were centrifuged at 3,000 rpm for 10 minutes and the supernatants were separated. The supernatants were heated on hot water at 97°C so as to evaporate and remove the ethanol. The residues were cooled and distilled water was added up to the same amount as before the evaporation of ethanol. Then, once again the solutions were centrifuged for 10 minutes at 3,000 rpm. The supernatants were separated and were used for the fermentation test as reported by HONMA et al. Thereby, the fermentation indices were determined. The procedure of separating the extract which was attempted occasionally is described below in each experiment.

Results

1. Extract from the Tissue of Normal Domestic Animals

Horse: Extracted solutions were prepared by the above described method from blood and from the tissues of marrow, liver, heart, spleen and brain. With each group of the solutions, fermentation experiments were performed. The fermentation indices obtained are shown respectively in table 1. The brain has the largest mean value of the indices followed by spleen, heart, and liver. Blood and marrow show comparatively small mean values. Statistical inspection denotes that the variances of the indices of brain and liver are larger than the others at the 1% level of significance. As for blood, marrow, heart and spleen, each variance belongs to that of the identical population. So statistical test of significance of the mean value difference shows that there is no difference between the spleen and heart, and between the blood and marrow. Further inspection makes it clear that the spleen and heart have larger mean value than the other two at the 1% level of significance.

Swine: Table 1 exhibits the results in reference to the extracted solution of the brain, of the liver and of the testis. It indicates that the variance and mean value of the fermentation indices of the brain are larger than the others. As to the testis and liver,

TABLE 1. *Extracted Solution from the Tissues of Domestic Animals*

DOMESTIC AMINAL	TISSUE	DEGREE OF FREEDOM	FERMENTATION INDEX (%)	
			Mean Value	Unbiased Estimate of Population Variance
Horse	Blood	9	80.7	2,922.0
	Marrow	6	82.6	8,333.2
	Liver	6	211.1	907,280.0
	Heart	10	264.3	14,727.6
	Spleen	5	265.1	6,713.4
	Brain	10	315.9	28,698.8
Cow	Brain	33	716.9	529,519.7
	Placenta	10	1,009.3	245,778.7
Swine	Liver	3	149.5	2,440.3
	Brain	42	432.7	42,457.1
	Testis	4	819.2	1,986.7

statistical inspection of the 2 mean values shows, at the 1% level of significance, that the testis has a larger mean value than the other. The mean value difference between the testis and brain cannot be tested statistically, because of inequality of variance of the population. As for the mean value of fermentation indices, the testis shows a value twice as large as that of the brain.

Cow: From the brain and placenta, extracted solutions were prepared. The results are in table 1. A difference between the mean values of the 2 fermentation indices is judged statistically at the 1% level of significance. That is to say, the placenta has contains more promoting substance than the brain.

Comparison of the cerebra of domestic animals: Table 2 shows each fermentation index of extracted solutions from the brain of 4 domestic animals. The indices of the extracted solutions from cow and sheep have larger variances and show statistically significant difference from the others, while there is no significant difference between the variance of horse and of pig at the 1% level of significance. Statistical inspection of

TABLE 2. *Extracted Solutions from the Brains of Domestic Animals*

DOMESTIC ANIMALS	DEGREE OF FREEDOM	FERMENTATION INDEX (%)	
		Mean Value	Unbiased Estimate of Population Variance
Horse	10	315.9	28,698.8
Swine	42	432.7	42,457.1
Cow	33	716.9	529,519.7
Sheep	8	787.4	230,680.9

each mean value difference is impossible because of inequality of the variances of the populations, but, if compared approximately, the ruminants have larger mean value than horse or pig.

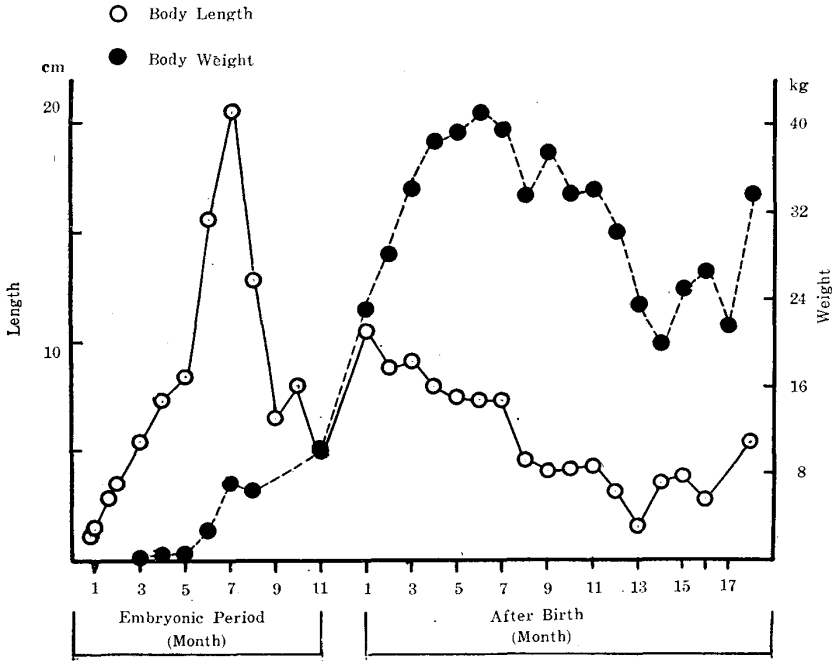
From the above results the following conclusions are drawn. In the tissues of domestic animals there is, in more or less amount, some substance which promotes yeast growth. The tissues of the placenta, of the brain, and of the testis include a relatively great amount of this promoting factor. Ruminants have a greater quantity of promoters in the brain than nonruminant animals.

2. Increase and Decrease of the Promoter in the Brain in Accordance with Growth

The solution extracted from the brain of embryos, of infants and of adults of normal cows and pigs was supplied for fermentation experiment. Investigations were performed about the relationship between the indices and the weight and the length which are indicators of growth. The length of embryo was determined by measuring from the crown to the hip, while a cow was measured from the shoulder to the hip.

The length and the weight of pig embryos are mean values of one farrow. The embryonic period was estimated collectively by the methods of CARPLET, SCHMALTZ, JUNG, OSAWA and SAKAI. The age of a cow was judged from the registry, teeth or by the horn-ring. As for pig, the age was estimated from teeth or from the breeder's information. Data of the weight and the length of cows ranging from birth to 18 months old were based on the survey of about 125 Holsteins which were surveyed from May, 1949 to January, 1958 at the Niikappu Breeding Farm attached to the Faculty of Agriculture of Hokkaido University.

FIG. 1. *Increase of Body Weight and Length per Month* (Cow)



Cow: Eighty-eight embryos and 29 cases of infants and adults were used. Changes of increase in the length and the weight are illustrated in fig. 1. The length of embryo increases uniformly until the 5th month, in the next 2 months it grows rapidly and reaches a peak at the 7th month. After that the increase of length rapidly goes down until the 11th month when it is about the same as the increase at the 3rd month. After birth the increase of length shows the highest point in the 1st month, and thereafter it gradually declines. A minimum increase is seen in the 13th month. From the 14th month it again goes up little by little.

As for the weight, it does not increase rapidly till the 5th month of the embryo and then the increase is relatively large. But at the 8th month it is retarded for a while, and it increases again up to the time of birth when a maximum increase of embryo weight appears. After birth, the weights augments rapidly and shows a maximum increase in the 6th month. After that, it agains falls until it reaches a minimum point in the 14th month after birth and then it goes up once again.

Fig. 2 and 3 indicate a transition of the fermentation indices. The indices continue increasing through the embryonic period. At the 3rd month after birth those indices increase abruptly and comes up to a peak in the 6th month; in the 12th month again they

FIG. 2. *Relation between Age and Fermentation Index of Bovine Brain* (Ranging from Embryonic Period to the 12th Month)

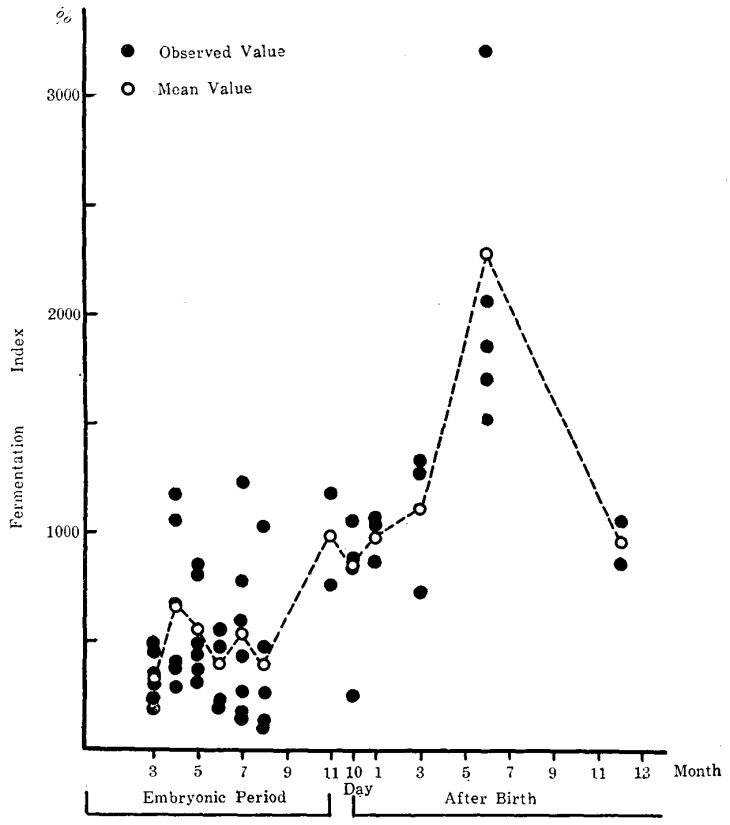
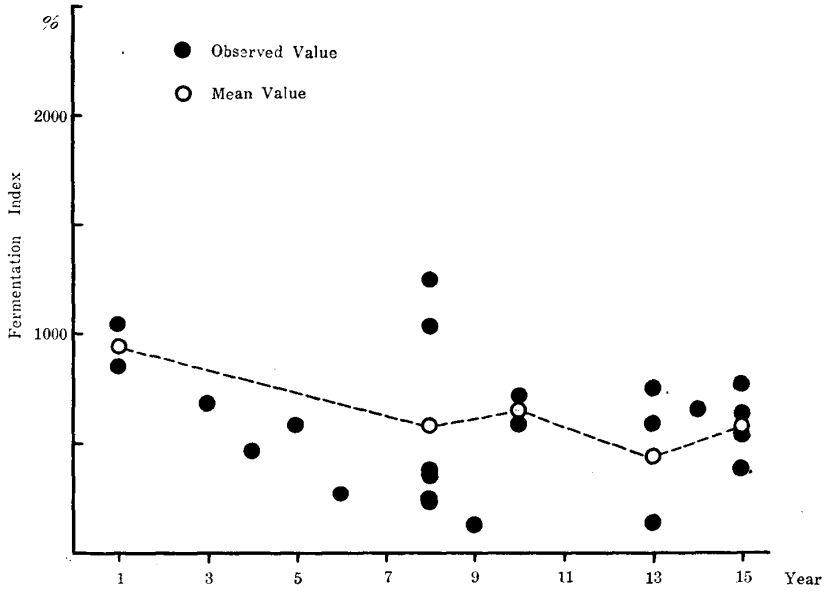


FIG. 3. *Relation between Age and Fermentation Index of Bovine Brain* (Ranging from the 12th Month to 180th Month)



drop down to the same figure as that of the 3rd month. After that no remarkable change can be observed.

Hereby it is clarified that the peak of the index in the 7th month corresponds to the peak of the weight increase.

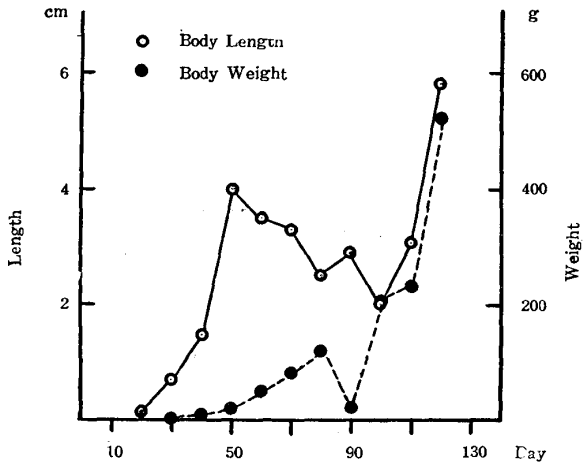
Swine: A hundred forty-five pigs in pregnancy, 1077 embryos and 40 adults were used for the experiment. The increase of length per 10 days is shown in fig. 4.

During the embryonic period, the increase of length reaches its 2nd height on the 50th day and comes down to a valley on the 100th day. Then it once again goes up abrupty to a maximum just before the parturition.

As for the weight increase a lower summit comes on the 80th day and a valley comes on the 90th day. After that time, the weight increase forms an upward line and reaches a maximum just before the birth. No observation was made about the growth after birth.

The fermentation indices were gained from extracted solutions by the aforementioned method from the homogenized brains of members of one farrow.

FIG. 4. *Increase of Body Weight and Length per 10 Days* (Swine)



As seen in fig. 5, in the embryonic period the index has a summit on the 50th day, then

FIG. 5. *Relation between Embryonic Period and Fermentation Index of Swine Brain*

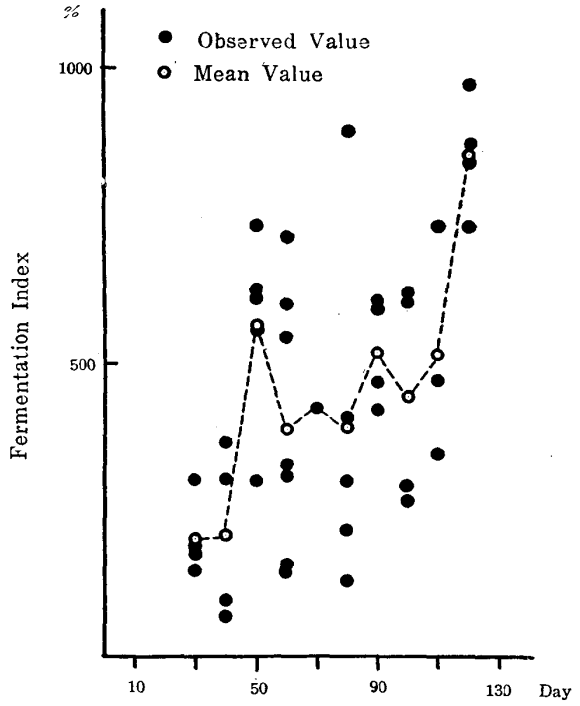


FIG. 6. *Relation between Age and Fermentation Index of Swine Brain*

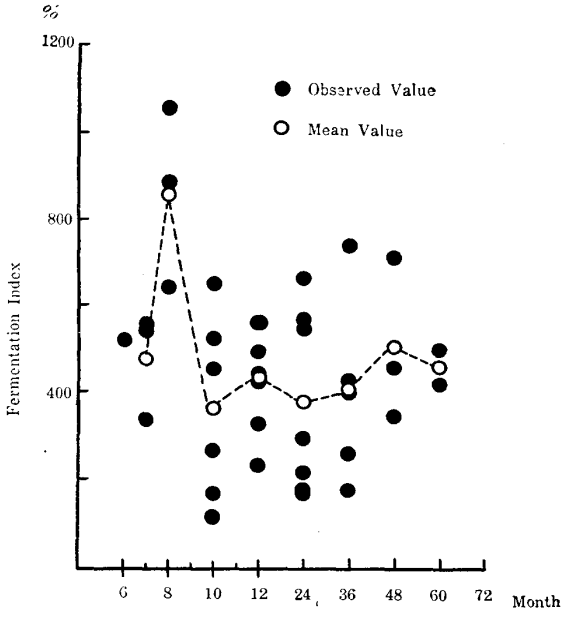
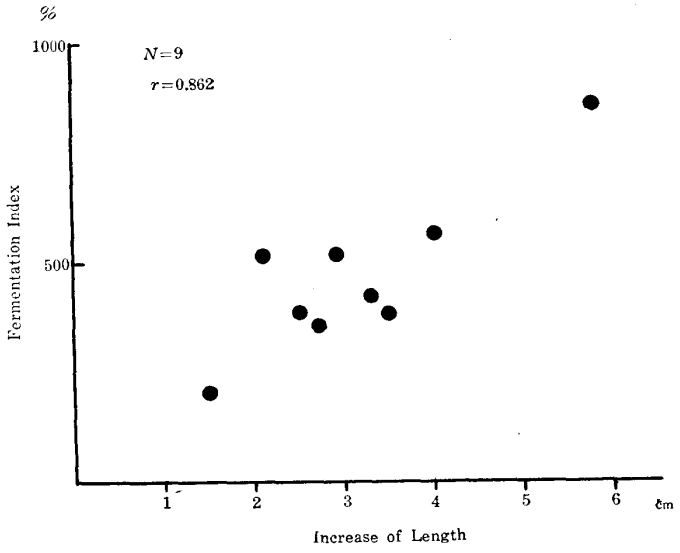


FIG. 7. *Correlation Diagram between Fermentation Index of Brain and Increase of Length in Embryonic Swine*



it decreases and next it abruptly increase after the 100th day until it shows a maximum just before the birth. This development of the index is quite similar to that of the length increase. Fig. 7 is a diagram of the correlation between the length increase and fermen-

tation index. The correlation coefficient is statistically enough, far beyond the 1% level of significance. After birth there is a hill at the 8th month, but no other conspicuous change can be observed, as seen in fig. 6.

The above observations prove it evident that there is some substance among the fermentation promoters extracted from the brain which increases or decreases in accordance with the growth.

3. Blood of Horse Suffering from Infectious Anemia

Blood samples of 49 horses suffering from infectious anemia were used. All these horses were slaughtered at Sapporo slaughter house from 1956 to 1958. Table 3 offers a comparison of the fermentation experiments on the extracted solutions from the sick horse blood and from the normal horse blood. As seen in the table, the mean value of fermentation indices and the variances are larger in the sick horse blood than in the normal horse blood.

TABLE 3. *Blood of Normal and of Horse Suffering from Infectious Anemia*

BLOOD	DEGREE OF FREEDOM	FERMENTATION INDEX (%)	
		Mean Value	Unbiased Estimate of Population Variance
Normal	9	80.7	2,922.0
Infectious Anemia	48	383.2	350,913.8

When the sick horses are classified into 3 groups of the normal temperature group (below 38°C), sub-fever group (ranging from 38°C to 39°C) and high fever group (above 39°C), it becomes clear, as seen in table 4, that the normal temperature group has the largest mean value of the fermentation index. Next follows the sub-fever group. The high fever group, of which samples were few, shows the smallest index. Besides, the order of the variances of these 3 groups is the same as the order of the mean values.

TABLE 4. *Blood of Infectious Anemia Horse*

CLINICAL STATE	DEGREE OF FREEDOM	FERMENTATION INDEX (%)	
		Mean Value	Unbiased Estimate of Population Variance
High Fever	2	189.0	69,944.5
Sub-Fever	34	323.0	158,852.9
Normal Temperature	10	627.5	1,749,824.8

4. Blood of Tuberculous Patients after Thoracoplasty

Blood was taken twice from 2 tuberculous patients in a hospital in Sapporo, the first time just before the operation, and the 2nd on the 54th day after the operation. From the samples, extracted solutions were prepared by the aforementioned method and a

fermentation experiments were performed. On the other hand, the patients urine was taken, just before the operation and 9 days after the operation, for the examination of the 4th urinary iodic acid reducing number (K_4) according to NISHIKASE's method.[18]

FIG. 8. *Fermentation Index of Blood and the 4th Iodic Acid Reducing Number of Urine of Tuberculous Patients*

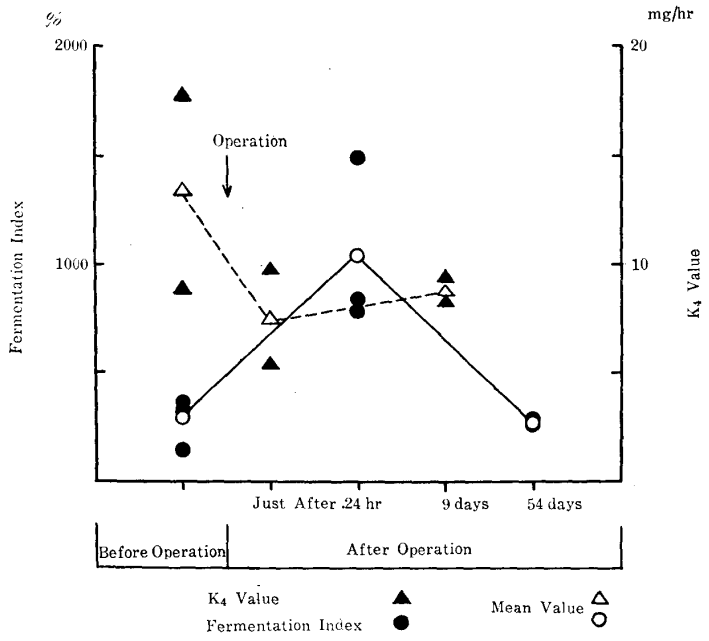
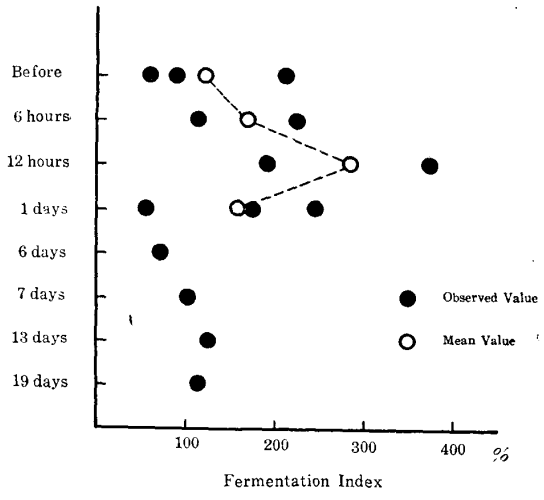


Fig. 8 shows the results. The fermentation index increases just after the operation, but 54 days later it is back again to the numerical value before the operation.

The K_4 value in the patients' urine is remarkably low just after the operation when compared with the value just before the operation and with that of 9 days after the operation.

FIG. 9. *Fermentation Index of Rabbit Blood at Low Pressure*



5. Blood of Rabbits Kept in the Low Pressure Chamber

Three normal healthy rabbits weighing 2 to 3 kg were used for the experiment. Blood samples were taken in the amounts of 3 to 8 ml and then animals were put in a glass vessel with a barometer. Next, air in the vessel was slowly pumped out for 15 minutes until the air pressure declined to 480 to 550 mmHg within the vessel. The rabbits were reared in this low pressure. Then blood was taken, about 3 to 8 ml, each time at the following times and days: 6

12, and 24 hours later and 7, 13 and 19 days later, respectively. The extracted solutions were prepared from the blood each time according to the author's method; they were employed for the fermentation test.

The results are shown in fig. 9. At 6 hours after the treatment, the fermentation promoter in blood increases remarkably and it goes up to the largest amount 12 hours later. Twenty-four hours later the increase again drops to the same value as at 6 hours. No great difference can be observed between the amount of the promoter existing on the 7th day and the amount before the treatment.

As seen in results reported in above three paragraphs, it is evident that the promoting factor, which is extracted by the author's method, varies in amount in accordance with such stresses as sickness, operation or low pressure.

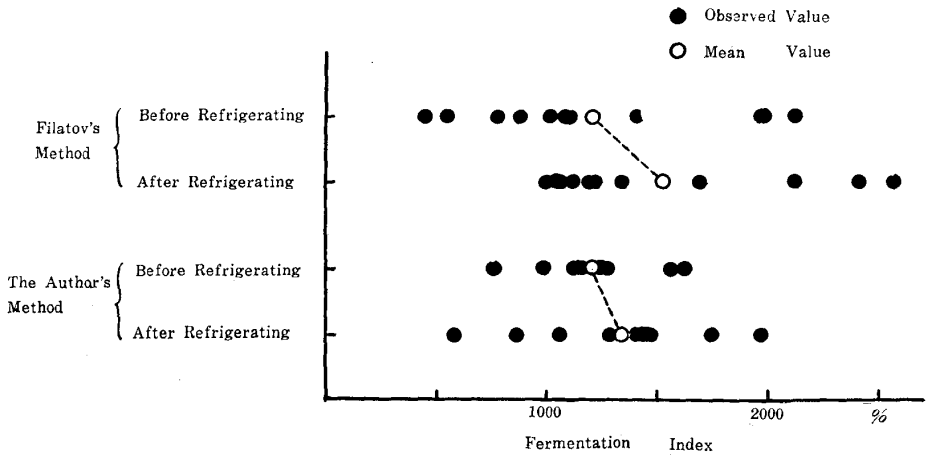
6. Reexamination of FILATOV's Biogenitic Stimulant

FILATOV reported that when the survived tissue was refrigerated at about 2°C, a biogenitic stimulant which acted to promote the process of fermentation of yeast was formed in the tissue. Reexamination was performed with bovine placenta and swine testis, while the extract from the refrigerated tissue prepared by the present author's method was compared with the biogenitic stimulant.

The solution of the biogenitic stimulant was prepared according to FILATOV's method. From a portion of the tissue after refrigerating, solution was extracted by means of the author's method. Moreover, just before refrigerating, solution was extracted by FILATOV's method or by the author's extracting method from a portion of the tissue.

Swine testis: The results are shown in fig. 10. The average value of the fermentation indices of the solution extrated by FILATOV's method after refrigerating increases in comparison with the value which shows before refrigerating. In statistical test, a difference of the 2 mean values is recognized at 1% level of significance. In case of the solution extracted by the author's method, the mean value of fermentation indices after refrigerating is larger than that before refrigerating and the variance of the fermentation

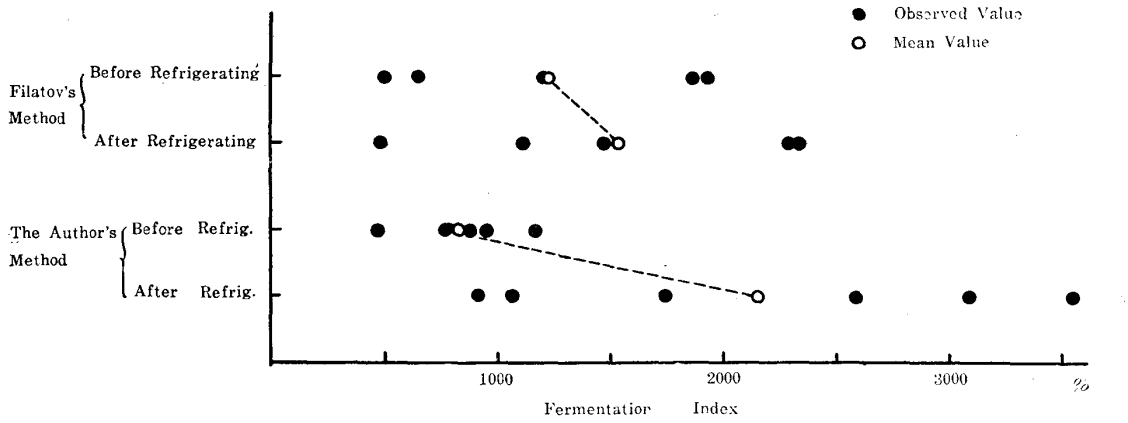
FIG. 10. *Extract from Swine Testis before and after Refrigerating*



indices before refrigerating is smaller than that after refrigerating.

Bovine placenta: As shown in fig. 11, in cases of the solution extracted by FILATOV's method or by the author's, the mean value of the fermentation indices is larger after refrigerating than before refrigerating. In case of material prepared by FILATOV's method, the difference between the mean value of the fermentation indices before refrigerating and that after refrigerating is indicated at 20% level of significance statistically. In case of prepared by the author's method, the variance is larger after refrigerating than before refrigerating at 1% level of significance. For this reason, the difference of the mean values can not be tested statistically.

FIG. 11. *Extract from Bovine Placenta before and after Refrigerating*



The above results indicate that the refrigerated tissue contains more biogenitic stimulant and more growth-promoting substance than the tissue before refrigerating.

7. The Solution Extracted from the Morbid Tissue

KAMEYAMA found that there was fermentation-promoting substance in the solution extracted from the morbid tissue. The author undertook to reexamine that statment employing material prepared by his own method. Use was made respectively of samples of each morbid material which was gotten from Sapporo slaughter house; *pneumonia crouposa* (swine), *pneumonia nectroticans et purulenta* (cow), *bronchopneumonia catarrhalis* (horse), *adenocarcinoma* (cow), *adenocarcinoma* (horse), *carcinoma hepatis* (horse), *seminoma* (horse) and icterus liver (horse). Each material was examined histopathologicaly and diagnosed in the Department of Pathology, Faculty of Veterinary Medicine, Hokkaido University. Each material was divided macroscopically into the following 3 parts: the normal part, the morbid part and the circumferential part. From each part, the growth-promoting substances were extracted and each extracted solution was used for fermentation test. The results, as shown in table 5, are the following: the materials which show larger fermentation index in the extracted solution from the morbid part than in that from the other parts are those derived from cases of *pneumonia crouposa* (swine) and *haemoangio-endothelioma* (cow); those which show the largest fermentation index in the circumferential parts are *pneumonia nectroticans et purulenta* (horse), *adenocavcinoma*

TABLE 5. *Fermentation Index of Morbid Tissue*

SAMPLE	FERMENTATION INDEX (%)		
	Morbid	Circumferent	Normal
Pneumonia crouposa (swine)	657	597	373
Haemoangio-endothelioma (cow)	1,253	933	—
Pneumonia necroticans et purulenta (cow)	54	160	20
Bronchopneumonia catarrhalis (horse)	150	196	146
Adenocarcinoma (horse)	15	910	800
Carcinoma hepatis (horse)	165	375	300
Adenocarcinoma (cow)	390	700	927
Seminoma (horse)	220	—	1,033
Icterus of Liver (horse)	0	—	211

(horse) and *carcinoma hepatis* (horse); those which indicate the largest fermentation index in the normal part are *adenocarcinoma* (cow), *seminoma* (horse) and icterus of liver (horse).

8. Qualitaive Chemical Character of the Growth Promoting Substance in the Solution Extracted by the Author's Method

Dialysis: A certain amount of the extracted solution of swine brain was poured into a cellophane bag. The bag was immersed for 24 hours in distilled water of 3 times quantity of the extracted solution and then, the inner and outer fluids were dried up. To each residue was added a certain amount of distilled water and then it was used for fermentation test. The results, as shown in table 6, indicate that the fermentation index of the solution in the bag decreased and that the growth promoting substance was recognized in the outer fluid. That is to say, the growth promoting substance is dialytic through cellophane membrane.

TABLE 6. *Dialysis of Extracted Solution from Swine Brain*

SAMPLE	FERMENTATION INDEX (%)
Extrated Solution	130
Outer Fluid	50
Inner Fluid	20

Effect of heat, acid and alkaline substances: The outer fluid as the extracted solution from swine brain was dialyzed against distilled water, was adjusted to pH 4.0~11.0 with 1/10 N HCl and with 1/10 N NaOH. Each solution was heated in the autoclave at about 120°C for an hour and then after being adjusted to pH 6.5, each one was used for the fermentation test. As shown in table 7, the growth promoting substance is scarcely

TABLE 7. *Influence of Heat and pH*

pH (at 120°C)	4	5	6	7	8	9	10	11	Original fluid (pH 5.6)
FERMENTATION INDEX (%)	130	130	160	100	160	120	160	140	190

destroyed within the limits of pH 6.0 and pH 11.0 at about 120°C.

Adsorption by acid clay, bone charcoal powder and Amberite IR 120: A certain quantity of the outer fluid obtained when the extracted solution from swine brain was dialyzed, was adjusted to pH 6.3 and acid clay or bone charcoal powder of 1/20 quantity of the fluid was added. The whole was stirred, warming on the water bath at about 50°C, for half an hour and then it was filtered through filter paper. This filtrate was adjusted to pH 6.5 and used for the fermentation test. The results, as shown in table 8, indicate that the growth promoting factor is hardly adsorbed to the acid clay but is considerably adsorbed to the bone charcoal powder.

TABLE 8. *Test of Adsorption by Acid Clay and Bone Chascoal*

SAMPLE	FERMENTATION INDEX (%)
Original fluid	628
Acid Clay	605
Bone Charcoal	414

The concentrated outer fluid was poured onto a column of Amberite IR 120 which is previously treated with 2N NaOH, with 2N HCl and with distilled water and then the adsorbed material was eluted with 2N HCl. The effiuent was collected and adjusted at pH 7.0 with 2N NaOH and then dried. The 1% solution of the dried material showed a large fermentation index and a high value in the K_4.

Paper chromatography: The above sample which was separated with Amberite IR 120 was developed on a filter paper (Toyoroshi No. 50) with acending one-dimensional chromatography. The solvent was n-butanol-acetic acid-water (4 : 1 : 2). When the colour development on the paper was performed with ninhydrin, a purple spot whose Rf was 0.15 could be recognized.

TABLE 9. *Relation between Fermentation Index and Nitrogen Content*

SAMPLES	NITROGEN CONTENT (mg/dl)	FERMENTATION INDEX (%)
Bovine brain (3 M)	57	1323
" (3 M)	54	1269
" (6 M)	75	1846
" (6 M)	77	1708
Blood of adult horse	14	125

Nitrogen content of the extracted solution: The nitrogen contents of the extracted solution from bovine brain and from the blood of horse were determined by KJELDAHL method. Then the nitrogen contents were compared with the fermentation indices. The results, as exhibited in table 9, indicated that the extracted solution which shows large value of the fermentation index has a high nitrogen content.

DISCUSSION

When the amount of the substance which promotes the growth of yeast is determined by means of the fermentation test which was developed by ISOGAWA or by HONMA et al., results bring out the following problem: It is necessary to distinguish whether only the yield of CO_2 gas is increased without the growth or multiplication of yeast being promoted, or whether the yield of CO_2 gas increases as a result of the growth or multiplication of yeast being promoted. In the former case, it may be decided that there is a fermentation-promoting substance and in the latter case, that there is a growth promoting substance. KAMEYAMA found that the fermentation index was difinitely proved to increase in proportion to the growth and multiplication of yeast. Therefore, it is almost certain that the fermentation index of the extracted solution in the present author's experiments is determined by the quantity of the growth promoting substance.

Such inorganic substances as Zn, Mn and Cu have been known as co-growth promoting factors of yeast[16]. Besides, it is regarded that even infinitesimal amounts of them are effective enough and that they may exist in the yeast suspended solution used for the experiment. KAMEYAMA found that the fermentation index became almost zero when the serum was burned to ashes. Therefore, the fermentation index in the author's experiment is considered to be slightly affected by the unknown inorganic substances. As the author's extracted solution contains vitamins and hormones which are known as growth promoting substances so far, the results should be cautionally interpreted except in the case of the experiment with the sample excluding relatively small quantity of impurities in which Amberite IR 120 was used.

If the extracted solution causes mingling of some substance inhibitory to the growth of yeast, the fermentation index is determined by the ratio of the inhibitor to promoter and it becomes difficult to estimate the quantity of the growth promoting substance from the fermentation index. Necrosin[10,15], one of the MENKIN's factors may be mentioned as a substance equivalent to the inhibitory substance, but it has not yet been really confirmed to check the growth of yeast. This problem should be solved in the future. In spite of the problem about the inhibitory substance, in this paper, considerations are presented supporting the idea that the fermentation index of the author's extracted solutisn indicate the

quantity of the growth promoting substance.

Since bios was discovered by WILIDIERS as the multiplication promoter of yeast, BOAS, EASTCOTT, WILLIAMS et al., KÖGL et al.,[12~14] OKAWAUCHI, ELVEHJEM et al. and GYÖRGY et al. reported that vitamins B_1, B_2, B_6, niacine, pantothenic acid, biotin and inositol also exist as the growth promoting factors of yeast. It is reported that vitamin B_1, B_2 and B_6 are easily destoryed by alkali, that biotin and pantothenic acid exist in the body in combination with macromolecule, and that B_1, B_2, B_6 and niacine are adsorbed into the acid clay in acid solution. The substance that the present author is seeking is not destroyed, even if it is heated when its pH value lies with the limits of pH 6.0 and 10.0. It is dialytic against cellophane membrane and is difficult to be adsorbed into the acid clay. Therefore, it is different from the vitamins which are known so far, except inositol.

NIELSEN and HARTELIVES reported that the amino acids: β-alanin, asparatic acid and glutamic acid have a growth promoting effect on yeast and that interrelation between vitamins and amino acids in respect to the action are complicated. In the author's experiment, the substance adsorbed into Amberite IR 120 showed positive reaction of a ninhydrin reagent but was distinguished from the amino acids because in paper chromatography the Rf differed from that of the amino acids. ACTH, a hormon, promotes the growth of yeast but it is not dialytic against cellophane membrane. Judging from these points, the substance promoting the growth of yeast which plays a principal role in the author's extracted solution is a different substance from the vitamins and hormones which are known so far, and it possesses a relatively low molecule which contains nitrogen. From the author's experiments, this substance always exists in the tissue of normal animals, especially much in the tissue of brain, testis and placenta and it increases in the blood in correspondence with such stresses as diseases in a moderate stage, low pressure and surgery operation. These facts indicate that the substance has relation to the protection reaction of the living body through some mechanism. KAMEYAMA found that the growth promoting substance increased in the serum of moderately diseased humans, but conversely decreased in the serum of a seriously diseased person. In the findings of the present experiments also, the promoting substance decreased in the blood of the high temperature group of horses suffering from infectious anemia, while it increases in the normal temperature group of those animals. KAMEYAMA estimated that the promoting substance was closely related with carbohydrate metabolism from the fact that the substance highly increased in cases of *diabetes mellitus* and *cirrosis hepatis*. If the growth promoting substance in the present experiment is regarded to increase in the stadium of alarm reaction or in stadium of resistance to a stress, but conversely to decrease in the stadium of exhaustion, it may be

concluded that KAMEYAMA's and the author's substances belong to the same category.

By the reexamination of FILATOV's biogenitic stimulant, the growth promoting substance was ascertained to increase in the refrigerated tissue. From this fact and from the experiment on the extracted solution from morbid tissue, it is estimated that the tissue affected by a stress yields the growth promoting substance locally.

FILATOV advocated that if a living body or the survived tissue is allowed to remain in a disadvantageous condition, the biogenitic stimulant yields in opposition to that condition. From this consideration, the growth promoting substance may have the same mechanism of production as the biogenitic stimulant. FILATOV named a mixture of organic acids and other organic substances as the biogenitic stimulant. Accordingly, the author's substance is possibly a component of the biogenitic stimulant. The growth promoting substance is not only found always in the normal tissue, but also it increases in the brain when the body is growing actively. Therefore, the substance is related to the growth of the animal body in some manner. It increases in the body when assimilation is excellent on the one hand and in accordance with stresses on the other hand. As the substance which was separated by Amberite IR 120 shows a large iodic acid reducing value as reported by NISHIKAZE[18~20], the substance may agree with one which NISHIKAZE pursues as the indicator of the body vitality. According to NISHIKAZE[21~24], the substance which shows iodic acid reducing reaction is closely related to the function of the adrenal gland and it decreases in the urine in accordance with all stresses such as disease, starvation, labour, coldness and fear. In the present experiment which investigated the fermentation index of the blood and the 4th iodic aicd reducing value of the urine of tuberculous patients, the iodic acid reduing value in the urine decreased when the fermentation index increased in the blood. Therefore, assuming that NISHIKAZE's and the present author's substance are the same, some factor which changes the function of the kidney in response to stress must be fuond. Accordingly, although both the author's and NISHIKAZE's substances are the positive substance of the iodic acid reaction, they are not considered to be the same substance. So the relation between the two substances is a subject to be investigated in the future.

SUMMARY

The yeast-growth promoting substance of the ethanol extracts from the normal and morbid tissues of domestic animals and from blood of human and rabbit affected by stresses, was investigated by means of the fermentation test. The obtained results were the following:

1. The growth promoting substance existed in blood and the tissue of marrow, liver, spleen, heart, brain, testis and placenta, there being especially much in the tissue of placenta, testis and brain.

2. The brain tissue of ruminants had larger quantity of the substance than that of non-ruminants.

3. The substance in the brain tissue of cow or of pig increased when the growth of the body was excellent.

4. The blood of the horse suffering from infections anemia had larger quantity of the substance than that of the normal horse, but the blood of the diseased horse with high fever had smaller quantity of the substance than that of the normal one.

5. Most samples of the morbid tissues had larger amount of the substance in the morbid part or in the circumferential part than in the normal part.

6. The substance in the excised tissue of bovine placenta and of swine testis increased after refrigrative storage.

7. The substance under discussion in the blood of the tuberculous patients increased temporarily after thoracoplasty, while the 4th iodic acid reducing reaction of the urine decreased temporarily after that operation.

8. The substance in the blood of rabbit increased temporarily after breeding at low pressure 480~550 mmHg. for 12 hours.

9. The substance was dialytic through cellophane membrane and stable against heat, acid and alkali.

10. The substance under investigation was adsorbed with difficulty onto the acid clay in the acid solution, while it was easily adsorbed onto Amberite IR 120. The adsorbed substance showed color reaction with ninhydrine reagent and also iodic acid reducing reaction. Developing with paper chromatography when the solvent was n-butanol-acetic acid-water 4 : 1 : 2, showed the Rf to be 0.15.

11. From the above experimental results, it was estimated that the growth promoting substance increases in the stadium of alarm reaction or in the stadium of resistance to a stress but decreases in the stadium of exhaustion in the animal body, that it is closely related with the growth of body and that it is a relatively low molecule which contains nitrogen which differs from vitamins and hormenes known already.

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