enamel, and this is expressed by the standard deviation of ± 0.015 g./100 g.

A second series of estimations was conducted on teeth which had resisted caries. The teeth used were sound premolars extracted because of paradontal disease or to clear an otherwise empty mouth, and came from the higher age groups. The values, again the means of three samples, are also shown in Table 1. With a mean of 0.083 g./100 g. enamel, and standard deviation of ± 0.021 g./100 g. there is no statistically significant difference between the nitrogen content of this enamel and that of the first series.

DISCUSSION

The values for enamel nitrogen found in the experiments reported here are lower than most reported to date. Bowes & Murray (1935) found 0.156 % in enamel separated by grinding it from the surface of the dentine. Deakins & Volker (1941) used the flotation method for separating enamel and dentine. From their figures expressed as protein with a nitrogen content assumed to be 16 % the range of nitrogen values was found to be 0.078–0.31 %. It is considered that the low results given in the present paper were obtained by reducing the dentine

impurity to a minimum. A simple comparison between the enamel-nitrogen content of the two groups of teeth gives little information. In this series at least two variants may operate. First, there is the possibility that age changes may influence nitrogen content. Secondly, on the basis of a proteolytic theory of dental caries there is a possible relationship between nitrogen content and caries susceptibility. Although the second series of estimations were conducted on teeth of proven caries resistance, the orthodontic premolars were of unknown susceptibility to caries, some would have survived, others would have succumbed. Further work is necessary to test the effect of these and other variants on enamel-nitrogen values.

SUMMARY

Nitrogen estimations carried out on the carefully separated enamel of sound premolar teeth give a mean value of 0.071 ± 0.015 % for the age group up to 16 years and 0.083 ± 0.021 % for older age groups. These values are lower than those previously reported.

My thanks are due to Dr Agnes Shore, Prof. W. R. Spurrell and Prof. M. A. Rushton for much helpful advice and criticism.

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Interrelationship of Certain Vitamins of the B Group in Aneurin, Riboflavin and Biotin Deficiencies

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(Received 1 November 1948)

A close interrelationship of vitamins of the B group may be expected, since they are fundamental to all forms of life, and are the controlling factors in the chain of oxidative removal of certain metabolites. Definite interrelationships among these vitamins have been established by investigations on human subjects and on animals. The treatment of multiple deficiencies in man with aneurin alone results in the development of pellagrous skin changes which disappear on administration of either nicotinic acid or

yeast (Lehmann & Nielsen, 1939, Salvesen, 1940; Braendstrup, 1940). Sydenstricker (1941) pointed out that, in deficiencies of the individual vitamins of the B group, treatment with one vitamin may be rapidly followed by the development of severe signs of deficiencies of others. Klopp, Abels & Rhoads (1943) found no clinical signs of riboflavin deficiency in subjects on normal diets receiving large doses of aneurin, but found an increased urinary excretion of riboflavin. Increased excretion of riboflavin in the urine in chronic and acute aneurin deficiencies was observed by Sure (1944). Unna & Clark (1942) were

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unable to show that administration of large amounts of the individual vitamins of the B group to rats deficient in one or more vitamins of this group aggravated symptoms of the specific deficiency. Sure & Ford (1942) observed a marked drop in the aneurin and riboflavin concentrations of organs and tissues of rats deprived of aneurin, while in riboflavin deficiency a drop in riboflavin content only was observed. Further, deprivation of aneurin interfered with riboflavin utilization; deprivation of riboflavin did not affect the utilization of aneurin. The efficiency of utilization of aneurin and riboflavin was also affected in deficiencies of these two vitamins (Sure & Ford, 1943). Similar studies (Russell & Nasset, 1941) indicated that pantothenic acid may play a role in the absorption of other B vitamins. The riboflavin concentration in the livers of rats deficient in one or more of the B vitamins increased during digestion and assimilation as a result of mobilization from other tissues (Supplee, Jensen, Bender & Kahlenberg, 1942). Pantothenic acid had a direct and specific function in this mobilization. Aneurin depletion was shown to diminish the process, while pyridoxine exerted no influence. Singher, Kensler, Levy, Poore, Rhoads & Unna (1944) have reported an increase in riboflavin concentration in the livers of aneurin-depleted rats, and an increase in aneurin concentration in the livers of riboflavin-deficient rats.

The present investigation was undertaken in order to study interrelationships among certain vitamins of the B group in aneurin, riboflavin and biotin deficiencies, and, secondly to find out whether these interrelationships hold good with enzymes, the prosthetic group of which is one or other of the B vitamins. The enzymes selected for this study were

described by Potter & Elvehjem (1936), kept cool by ice water. The homogenized solution was passed through fine cloth and the residue homogenized again. Final volumes of the suspension were so adjusted that 1 ml. contained 100 mg. of the wet tissue. The suspension was kept in ice between experiments and only used during the day of its preparation. The hearts of a number of rats were pooled to obtain enough suspension for a set of experiments. In every experiment the weight of the dried tissue was determined.

General methods. Livers from the experimental and control rats were used for the estimation of vitamins. Aneurin was estimated by a thiochrome method (Bhagvat, 1943), riboflavin fluorimetrically and nicotinic acid by the cyanogen bromide method of Swaminathan (1942*a, b*).

The activities of the enzymes, aldehyde oxidase, lactic and succinic dehydrogenases, were estimated by the method of Thunberg (1920) with methylene blue as the hydrogen acceptor. The reactions were carried out at 37° in ordinary test tubes into which purified melted vaseline was poured over the reaction mixture. Owing to the absence of Thunberg tubes due to war conditions, it was necessary to devise an alternative technique to obtain anaerobic conditions. Various methods were tried, the most reproducible results being obtained with melted vaseline, which solidified in a few seconds and acted as a seal, thereby establishing anaerobic conditions. The results of this technique were comparable with those obtained when Thunberg tubes were used. The different solutions were added in the order: substrate, KCN, buffer, water, enzyme suspension and methylene blue. The tubes were shaken, and after the addition of melted vaseline were left at 37°. The time required for the decolorization of methylene blue was noted. Experiments were always set up in duplicate and with proper controls. The activity of the enzyme is expressed by the volume of O₂ (in μ l.) equivalent to methylene blue reduced in the presence of substrate in 1 hr. by 1 mg. of dried tissue.

Preliminary trials were carried out to find out the optimum conditions for the activity of the various enzymes. The relative amounts of the substrate, enzyme and methylene blue used in these studies are given in Table 1.

Table 1. *Optimum conditions for measurement of enzyme activities*

Enzyme	Suspension (ml.)	KCN m/15 (ml.)	Methylene blue (0.1%) (ml.)	Substrate	Water (ml.)	Phosphate buffer (pH 7.4) (ml.)	Final volume (ml.)
Aldehyde oxidase	1	—	0.4	0.1 ml. M-acetaldehyde	0.5	1.0	3
Lactic dehydrogenase	1	0.4	0.2	0.1 ml. M-sodium lactate	0.3	1.0	3
Succinic dehydrogenase	0.5	—	0.2	0.2 ml. M-sodium succinate	2.1	1.0	4

the aldehyde and pyruvic oxidases and the lactic and succinic dehydrogenases. The effect of deficiency of certain members of the B group of vitamins on the activities of these enzymes has been investigated.

EXPERIMENTAL

Estimation of enzymic activity of tissues

Preparation of tissue suspension. The organs (liver, kidney and heart) from freshly killed rats (rats were killed by a blow on the head) were dissected out and weighed in an ice-cold watch glass. They were ground in an ice-cold mortar with cold 0.1 M-phosphate buffer (pH 7.4). The suspension was homogenized for 2-3 min. in a homogenizer of the type

KCN was incorporated in the lactic dehydrogenase system to prevent inhibition of this enzyme by pyruvic acid, formed by oxidation (Green & Brosteaux, 1936).

The activity of the pyruvic oxidase was determined by the ability of the tissue to oxidize pyruvic acid *in vitro*. The homogenized tissue suspension (1 ml.) was incubated with 50 mg. of pyruvic acid at 37° for 1 hr. The pyruvic acid remaining in the reaction mixture was estimated by the method of Lu (1939). The results are expressed as mg. of pyruvic acid removed by 100 mg. dried tissue in 1 hr.

Animal experiments

Young rats weighing 45-65 g. were caged individually over a wide mesh screening and were fed *ad lib* on the

Table 2. *Composition of the experimental diets*

Component	Aneurin deficient (%)	Riboflavin deficient (%)	Biotin deficient	
			Experimental (%)	Control (%)
Sucrose (purified)	58.0	58.0	54.0	54.0
Casein (purified)	18.0	18.0	15.0	15.0
Salt mixture* (cf. McCollum & Davis, 1913)	4.0	4.0	4.0	4.0
Autoclaved brewer's yeast	10.0	Nil	Nil	Nil
Alkali-autoclaved brewer's yeast	Nil	10.0	Nil	Nil
Dried raw egg white	Nil	Nil	15.0	Nil
Dried autoclaved egg white	Nil	Nil	Nil	15.0
Gingelly oil	8.0	8.0	8.0	8.0
Dried ox liver	Nil	Nil	2.0	2.0
Shark-liver oil	2.0	2.0	2.0	2.0
	100.0	100.0	100.0	100.0

Vitamin supplements/rat/day

	Aneurin deficient (μg.)		Riboflavin deficient (μg.)		Biotin deficient (μg.)	
	Control	Experimental	Control	Experimental	Control	Experimental
Aneurin	5	Nil	20	20	20	20
Riboflavin	Nil	Nil	45	Nil	45	45
Pantothenic acid	Nil	Nil	100	100	100	100
Pyridoxine	Nil	Nil	20	20	20	20

* The composition of the salt mixture used was as follows: calcium lactate (39.00 g.), calcium phosphate (16.20 g.), potassium phosphate (28.62 g.), iron citrate (3.54 g.), sodium chloride (5.19 g.), magnesium chloride (7.98 g.), sodium phosphate (10.41 g.), potassium iodide (0.50 g.).

different basal diets. The rats were distributed equally between the two sexes. The composition of the different basal diets used is shown in Table 2.

Sucrose was purified by repeated washings with 95% ethanol. Casein was purified by repeated solution in alkali and precipitation with dilute acid. The precipitated casein was washed free from acid and then with ethanol and dried in the sun. The dried casein was refluxed with 95% ethanol for 24 hr.

For experiments on aneurin deficiency, autoclaved yeast was prepared by autoclaving brewer's yeast at 15 lb. pressure for 6 hr. in layers not exceeding $\frac{1}{4}$ in. and subsequently drying in the sun. For riboflavin deficiency studies, dried brewer's yeast was moistened with water and brought to pH 11.0. The wet mass, spread in thin layers, was then autoclaved at 15 lb. pressure for 6 hr. and dried in the sun.

Egg white was prepared from hens' eggs and was dried in the sun. Autoclaved egg white was prepared by autoclaving hens' eggs at 15 lb. pressure for 30 min. and subsequently drying in the sun. This treatment inactivated avidin, the antibiotic principle of egg white.

The different experimental diets were mixed and given to the animals in small trays. The experiments were repeated on three separate batches of 60 rats. The positive controls, i.e. those receiving all the vitamins, grew steadily while the negative controls grew for a time, then lost weight and developed symptoms characteristic of the particular deficiency under experiment. The time required for the onset of these symptoms varied in different experiments. Thus, the symptoms of aneurin deficiency manifested themselves in 3-4 weeks, those of riboflavin deficiency in 6-8 weeks and those of biotin deficiencies in 8-10 weeks. The animals were kept on the deficient diets till severe signs of deficiency developed. Deficient and control animals were sacrificed simultaneously and their tissues analysed for their vitamin content and enzymic content by the methods described above.

RESULTS

The effect of aneurin, riboflavin and biotin deficiencies on the aneurin, riboflavin and nicotinic acid concentrations in rat livers was studied. The relevant data are presented in Table 3.

Significant differences in the vitamin contents of the liver were observed when rats were fed the different experimental diets. Depletion of aneurin was found to increase the concentration of riboflavin in the liver above that of control animals, while in riboflavin deficiency concentration of aneurin in the liver was higher than in control animals. These observations confirm the findings of Singher *et al.* (1944). The aneurin and riboflavin concentrations in the livers of the rats maintained on diets deficient in the respective vitamins decreased rapidly during the deficiency period. Livers of aneurin- and riboflavin-deficient rats contained smaller amounts of nicotinic acid than the livers of control animals. Aneurin, in the liver of the rats maintained on diet deficient in biotin, showed marked decrease. A considerable difficulty was experienced in aneurin estimation in these series of experiments owing to the presence in the liver of certain fluorescent substances. The interference encountered was greater with deficient livers, while riboflavin showed an increase over that present in the livers of the control animals. Singher *et al.* (1944) did not find any significant difference in the concentrations of aneurin and riboflavin in the livers of control and biotin-deficient rats.

Table 3. *Effect of aneurin, riboflavin and biotin deficiencies on the aneurin, riboflavin and nicotinic acid content of rat livers*

Deficiency		Aneurin ($\mu\text{g./mg.}$)		Riboflavin ($\mu\text{g./mg.}$)		Nicotinic acid ($\mu\text{g./g.}$)	
		Range	Mean	Range	Mean	Range	Mean
Aneurin	Control	2.00-7.00	3.66	13.50-28.30	21.56	48.30-100.00	75.26
	Deficient	0.40-1.60	0.83	16.30-43.00	27.70	36.30-85.70	63.94
Riboflavin	Control	1.60-8.00	4.72	15.00-35.30	22.66	29.00-150.00	75.24
	Deficient	3.60-15.50	6.88	5.00-13.10	9.76	42.80-94.00	61.30
Biotin	Control	0.80-5.50	2.14	19.80-34.80	27.77	25.00-155.00	60.78
	Deficient	0.00-2.00	0.69	25.90-49.50	37.12	37.00-120.00	59.18

Statistical analysis of the results

Vitamin		Mean (mg./g.)*		Difference of means (a) - (b)	Statistical ratio (d or t)†	5% level of significance of the statistical ratio	Remarks
		Control (a)	Deficient (b)				
Aneurin series	Aneurin	3.66 (18)	0.83 (24)	2.83	8.98 (d)	2.10	Significant
	Riboflavin	21.56 (18)	27.70 (43)	6.14	2.97 (t)	2.00	"
	Nicotinic acid	75.26 (17)	63.94 (25)	11.32	2.31 (t)	2.02	"
Riboflavin series	Aneurin	4.72 (46)	6.88 (37)	2.16	4.30 (d)	1.97	"
	Riboflavin	22.66 (34)	9.76 (35)	12.90	14.09 (d)	1.97	"
	Nicotinic acid	75.24 (39)	61.30 (33)	14.00	2.01 (d)	1.97	"
Biotin series	Aneurin	2.14 (24)	0.69 (24)	1.45	5.8 (d)	2.06	"
	Riboflavin	27.77 (24)	37.12 (24)	9.35	5.66 (t)	2.02	"
	Nicotinic acid	60.78 (22)	59.18 (20)	1.60	0.18 (t)	2.02	Not significant

* The figures in brackets represent the number of experimental animals.

† $d = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{(s_1^2 + s_2^2)}}$, where $\bar{X}_1$ and $\bar{X}_2$ are the two mean values, and s_1^2 and s_2^2 are the respective standard errors of the means. $t = \frac{\bar{X}_1 - \bar{X}_2}{s}$; s is the error of the difference calculated on the assumption that the standard deviations of the observations within the control group and the deficient group are not significantly different from each other. The letters (d) and (t) in brackets in this column represent the formula used for calculating the statistical ratio.

The activities of certain enzymes of rat tissues in aneurin, riboflavin and biotin deficiencies were next investigated. The results are illustrated in Tables 4 and 5.

Marked variations have been observed in the aldehyde oxidase content of livers and that of lactic dehydrogenase content of liver and kidneys of control rats in the three experiments. The control rats in the first experiment (aneurin deficiency) received only aneurin as a supplement, while in the other two experiments (riboflavin and biotin deficiencies respectively) in addition to aneurin, other vitamins, viz. riboflavin, pyridoxine and pantothenic acid, were given as supplements to control rats. The addition of these vitamins appears to influence the concentrations of the aldehyde

oxidase and the lactic dehydrogenase in the liver and that of lactic dehydrogenase in the kidney of rats.

In the livers from aneurin-deficient rats, the activity of the aldehyde oxidase was only 60% of that found in control rats. The activity of the succinic dehydrogenase in deficient tissues, however, was found to increase by 22% in the liver, 18% in the kidney and 42% in the heart over that found in control rats. Axelrod, Potter & Elvehjem (1942) have suggested that riboflavin is the prosthetic group of succinic dehydrogenase. Since in rats the depletion of aneurin was found to increase the concentration of riboflavin in the liver (heart and kidney were not analysed for their vitamin content) the increased succinic dehydrogenase activity might be attributed to the availability of bigger amounts of

Table 4. *Effect of aneurin, riboflavin and biotin deficiencies on the activities of certain enzymes of rat tissues*
(Results are expressed as mol. O₂ equivalent to methylene blue reduced in 1 hr./mg. of dried tissue.)

Deficiency	Liver			Kidney			Heart		
	Aldehyde oxidase		Lactic dehydrogenase	Succinic dehydrogenase		Lactic dehydrogenase	Succinic dehydrogenase		Succinic dehydrogenase
	Range	Mean		Range	Mean		Range	Mean	
	Control	Deficient	Control	Deficient	Control	Deficient	Control	Deficient	Control
Aneurin	0.42-2.27	1.12	0.82-4.9	1.66	0.86-6.23	2.77	1.12-2.92	1.96	3.13-8.04
Riboflavin	0.29-2.01	0.86	0.75-2.69	1.47	1.05-7.33	3.27	0.97-3.66	2.18	5.01-8.83
Biotin	1.45-10.07	5.49	0.79-14.87	8.9	2.16-10.34	5.25	1.73-13.98	6.79	3.6-9.24
	0.65-3.2	1.54	0.87-3.5	1.78	1.9-5.8	3.46	0.8-4.2	2.65	1.5-5.7
	1.5-17.7	6.93	2.6-46.6	6.67	2.1-8.1	3.33	2.3-16.4	9.36	2.7-5.2
	2.7-12.6	5.5	1.0-13.8	12.92	2.1-5.1	3.96	2.1-12.7	9.06	2.3-6.6
Statistical analysis of the results									
	Aneurin series			Riboflavin series			Biotin series		
	Mean*	Difference of the means	Statistical ratio (d or t)†	Mean*	Difference of the means	Statistical ratio (d or t)†	Mean*	Difference of the means	Statistical ratio (d or t)†
	Control	Deficient	Control	Deficient	Control	Deficient	Control	Deficient	Control
	1.12 (31)	0.86 (53)	0.26	2.10 (t)	1.99	Significant	5.49 (45)	1.54 (33)	9.83 (d)
Liver aldehyde oxidase	1.66 (34)	1.47 (33)	0.19	1.06 (t)	2.06	Not significant	8.90 (45)	1.78 (30)	10.74 (d)
Liver lactic dehydrogenase	2.77 (44)	3.27 (62)	0.50	2.17 (t)	1.99	Significant	5.25 (46)	3.46 (33)	5.04 (t)
Liver succinic dehydrogenase	1.96 (37)	2.18 (51)	0.22	1.40 (d)	2.03	Not significant	6.79 (47)	2.65 (31)	6.11 (d)
Kidney lactic dehydrogenase	4.94 (37)	5.37 (51)	0.83	2.77 (t)	1.99	Significant	5.20 (47)	2.94 (32)	7.24 (t)
Kidney succinic dehydrogenase	4.89 (10)	6.78 (10)	1.89	2.52 (t)	2.10	Significant	3.31 (18)	0.93 (8)	5.59 (d)
Heart lactic dehydrogenase							5.61 (18)	3.00 (10)	6.37 (t)
Heart succinic dehydrogenase									
	Liver			Kidney			Heart		
	Mean*	Difference of the means	Statistical ratio (d or t)†	Mean*	Difference of the means	Statistical ratio (d or t)†	Mean*	Difference of the means	Statistical ratio (d or t)†
	Control	Deficient	Control	Deficient	Control	Deficient	Control	Deficient	Control
	6.93 (28)	5.5 (29)	1.43	3.40 (t)	2.00	Significant	5.49 (45)	1.54 (33)	9.83 (d)
Liver aldehyde oxidase	7.67 (23)	12.92 (28)	5.25	2.98 (d)	2.06	Not significant	8.90 (45)	1.78 (30)	10.74 (d)
Liver lactic dehydrogenase	3.33 (29)	3.96 (27)	0.63	1.96 (d)	2.06	Not significant	5.25 (46)	3.46 (33)	5.04 (t)
Liver succinic dehydrogenase	9.36 (28)	9.06 (28)	0.30	0.25 (d)	2.06	Not significant	6.79 (47)	2.65 (31)	6.11 (d)
Kidney lactic dehydrogenase	4.04 (27)	4.03 (28)	0.01	0.03 (t)	2.00	Not significant	5.20 (47)	2.94 (32)	7.24 (t)
Kidney succinic dehydrogenase	2.40 (8)	3.37 (12)	0.97	1.23 (d)	2.18	Not significant	3.31 (18)	0.93 (8)	5.59 (d)
Heart lactic dehydrogenase	4.12 (8)	4.12 (12)	0.40	0.82 (t)	2.10	Not significant	5.61 (18)	3.00 (10)	6.37 (t)
Heart succinic dehydrogenase									

* The figures in brackets represent the number of experimental animals.

† $d = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{(s_1^2 + s_2^2)}}$, where $\bar{X}_1$ and $\bar{X}_2$ are the two mean values, and s_1^2 and s_2^2 are the respective standard errors of the means. $t = \frac{\bar{X}_1 - \bar{X}_2}{s}$, s is the error of the difference calculated on the assumption that the standard deviations of the observations within the control group and the deficient group are not significantly different from each other. The letters (d) and (t) in brackets in this column represent the formula used for calculating the statistical ratio.

Table 5. *Effect of riboflavin and biotin deficiencies on the pyruvic oxidase activity of rat liver*

		Pyruvic acid (μ g.) removed by 100 mg. dried liver/hr.				
Deficiency		Range	Mean			
Riboflavin	Control	122-90-183-10	150-28			
	Deficient	75-10-137-00	103-30			
Biotin	Control	129-50-195-50	154-73			
	Deficient	112-00-183-00	142-24			

Statistical analyses of the results						
	Mean*		Difference of the means	Statistical ratio (t)†	5% level of significance of statistical ratio	Remarks
	Control	Deficient				
Riboflavin series	150-28 (20)	103-30 (20)	46-98	8-34	2-02	Significant
Biotin series	154-73 (29)	142-22 (22)	12-49	2-81	2-01	"

* The figures in brackets represent the number of experimental animals.
 † $t = \frac{\bar{X}_1 - \bar{X}_2}{s}$; s is the error of the difference calculated on the assumption that the standard deviations of the observations within the control group and the deficient group are not significantly different from each other.

the prosthetic group. The activity of the lactic dehydrogenase in the livers of deficient rats did not vary significantly from that of livers of control rats.

In riboflavin deficiency, the activities of all the enzymes in the liver, kidney and heart were found to be markedly affected. Thus, in the livers, the activities of aldehyde oxidase, succinic and lactic dehydrogenases were only 18, 60 and 20% respectively of those found in the control animals. The activities of lactic and succinic dehydrogenases of the heart and kidney were also found to be significantly decreased. This diminution might be regarded as a result of the lowered amount of the prosthetic group (riboflavin) available. A similar fall in the activity of enzymes with riboflavin as the prosthetic group, e.g. xanthine oxidase (Ball, 1938, 1939; Corran, Dewan, Gordon & Green, 1939), succinoxidase and D-amino-acid oxidase (Axelrod & Elvehjem, 1941; Axelrod, Potter & Elvehjem, 1942; Axelrod, Swingle & Elvehjem, 1942; Axelrod, Sober & Elvehjem, 1939), has been reported. The effect of riboflavin deficiency on the concentration of coenzymes and other factors necessary for the complete oxidation of certain metabolites, viz. aldehyde, lactate, etc. was not investigated.

In biotin deficiency the oxidation of aldehyde by the liver was decreased below that of control rats, while that of lactate was increased. The succinic and lactic dehydrogenases of heart and kidney were found to be unaffected in biotin deficiency. This confirms with the findings of Axelrod *et al.* (1939). The pyruvic oxidase activity of rat liver in ribo-

flavin and biotin deficiency was decreased. This might be due to partial depletion of the two vitamins in the tissues, since it has been reported that riboflavin and biotin are essential for the complete oxidation of pyruvic acid (Lipmann, 1939; Pilgrim & Elvehjem 1944; Pilgrim, Axelrod & Elvehjem, 1942).

In the oxidation of acetaldehyde, all the three vitamins appear to be involved, since withdrawal of any one of these decreases the activity of the aldehyde oxidase, though its prosthetic group has been shown to be riboflavin (Subrahmanyam, Gordon & Green, 1939).

The results in general show that the activity of the different enzymes studied depends on the relative concentrations of the different B vitamins, thereby indicating a close interrelationship between them.

SUMMARY

1. Interrelationships between the vitamins of the B group in rats in aneurin, riboflavin and biotin deficiencies have been studied.
2. The riboflavin concentration in the liver was found to be increased in aneurin-deficient rats, while riboflavin-deficient rats had an increased concentration of aneurin in the liver. In biotin deficiency, the concentration of riboflavin in the liver was increased, while that of aneurin decreased. Livers from aneurin-deficient and riboflavin-deficient rats contained a smaller amount of nicotinic acid than that present in livers of control rats.
3. The activities of aldehyde oxidase, lactic and succinic dehydrogenases depends on the relative

concentration of aneurin, riboflavin, nicotinic acid and biotin in the tissues. Increase in the concentration of riboflavin in the liver increases the activity of the lactic and succinic enzymes (with the exception of lactic dehydrogenase of aneurin-deficient livers), while a decrease in the riboflavin content decreases their activity. Similar results have been obtained for lactic and succinic dehydrogenases of heart and kidney in riboflavin series and for succinic dehydrogenase in aneurin series; while the enzyme make up of these tissues has been shown to be unaffected in biotin series.

4. The oxidations of acetaldehyde and pyruvic acid appear to involve all the vitamins, aneurin, riboflavin and biotin, since withdrawal of any one of these appears to decrease the activities of their respective enzymes.

5. The results are interpreted as evidence of an interrelationship between certain vitamins of the B group.

The authors are grateful to Mr S. J. Poti of the Department of Statistics, All India Institute of Hygiene and Public Health, Calcutta, for the statistical analysis of the results presented in the paper.

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The Fractionation of Weak Electrolyte Mixtures by Ion-Exchange Resins

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(Received 31 December 1948)

Ion-exchange resins are becoming of increasing importance in the separation of mixtures of weak electrolytes, and the object of the present paper is to enumerate some general considerations which are of importance in the planning and interpretation of separation experiments.

Choice of resin. Numerous experiments in these laboratories and elsewhere (see, for instance, Partridge* & Brimley, 1949) have established the fact that processes which involve the ionization of weakly acidic or basic groups in the resin structure are, com-

paratively, very slow; they also involve large swelling changes. The reverse processes are equally slow, probably because it is the surface of the granules that will be first attacked by a fresh reagent, and a reduction of charge and a desorption of water in the surface layers will hinder the diffusion of material to and from the interior of the gel. Procedures involving an alteration in the charge of the resin are therefore not well suited to chromatographic separations. For the types of resin in common use this means that cationic exchangers containing phenolic as well as sulphonic acid groupings should not be used at pH values greater than 8, and that the weakly acidic or weakly basic resins should if possible be employed

* The author is much indebted to Dr Partridge for an early view and discussion of his recent papers.