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EFFECT OF DIET-INDUCED THIAMINE DEFICIENCY ON VISCERAL DNA SYNTHESIS AND TISSUE COMPOSITION*

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Abstract—The aims of this study were to assess the effect of dietary thiamine deficiency in rats on DNA synthesis and DNA, RNA and protein concentrations of heart, kidney, pancreas and liver. DNA synthesis rates, as determined by [^{14}C]thymidine incorporation, declined in thiamine-deficient (TD) rats with neurologic signs to 36, 52, 32 and 64 per cent of pair-fed control (PFC) values in heart, kidney, pancreas and liver, respectively, 180 min after an intraperitoneal injection of the isotope ($P < 0.05$). Within 48 hr of thiamine repletion ("reversal"), DNA synthesis increased markedly in these organs of previously thiamine-depleted rats to 662, 3200, 307 and 1600 per cent of PFC values ($P < 0.05$). A 48-hr fast, in which TD and PFC food intake was restricted to 1 g/24 hr, after thiamine repletion, blunted the rebound in DNA synthesis. However, the DNA synthesis TD/PFC ratios in the reversed TD rats were still higher than during the symptomatic stage ($P < 0.05$), even in the presence of food restriction. Alterations in DNA labeling were not caused by the hypothermia which accompanies thiamine deficiency, and the ^{14}C-label was located in the DNA thymine moiety in both TD and control groups. All organs, except the kidney, weighed less in symptomatic TD rats, and except for the heart, this effect was fully reversed within 48 hr of thiamine repletion. Total organ DNA was significantly reduced only in the liver, while DNA concentration (mg/g of tissue) in TD rats was significantly higher in heart, pancreas and liver. Protein concentration was generally comparable in TD and PFC tissues, and total RNA concentration was slightly lower only in the TD liver. In conclusion, dietary thiamine deficiency resulted in decreased DNA synthesis in every tissue studied and this appeared to involve only a small DNA pool. Since this type of experimental thiamine lack is accompanied by anorexia and weight loss which cannot be fully compensated by pair feeding, it is uncertain if the changes in DNA synthesis and tissue composition are due to thiamine deprivation *per se* or to secondary nutritional effects of thiamine deficiency such as impaired food utilization.

Thiamine deficiency is commonly found in chronic alcoholics and, in its severe form, may lead to cardiac and neurologic dysfunction [1, 2]. The specific mechanisms for these disorders have not been elucidated as yet. Studies of various animal models of thiamine deficiency, however, have clearly shown various enzymatic abnormalities. The principal derangement is a reduction in transketolase activity [3, 4], which has an important role in the hexose monophosphate shunt [5]. Prior studies have shown that thiamine deficiency depresses the hexose monophosphate (HMP) shunt in erythrocytes [6] and intestinal mucosa [7]; however, the effect of decreased transketolase activity on the overall HMP pathway in other tissues is uncertain. The other enzymes which are impaired in thiamine deficiency (albeit to a lesser degree) are pyruvate and α-ketoglutarate decarboxylases [4, 8], wherein thiamine is a necessary cofactor. These enzymes contribute importantly to the viability of the citric acid cycle. Since these two pathways supply vital precursors (ribose, ATP) to nucleotide synthesis, a previous study was undertaken in this laboratory [9] to determine the effect of dietary thiamine deficiency on rat brain RNA and DNA synthesis. RNA synthesis was unaffected but the formation of at least one pool of DNA was seriously impaired. The present study is an expansion of our prior work to include an evaluation of the effect of thiamine deficiency on DNA synthesis rates, on DNA, RNA and protein levels, and on organ weights of heart, kidney, pancreas and liver.

MATERIALS AND METHODS

Animal model and study protocol

Female Sprague–Dawley rats, 60–80 g, were obtained from Harlan Industries, Inc., Indianapolis, Ind., in sets of three littermates. One animal in each set was fed a thiamine-deficient diet (Teklad Mills, Madison, Wisc.)† *ad lib.*, a second was pair-fed the same quantity of diet containing thiamine, and the third was given a thiamine replete diet *ad lib.* Water was supplied *ad lib.* Rats fed the thiamine-free diet showed neurologic dysfunction (ataxia, incoordination) after 4.5 weeks. These signs could be reversed within 6 hr of a single i.p. injection of 500 μg thiamine. In the following studies, "symptomatic" refers to thiamine-deficient rats showing neurologic dysfunction. Neither control group showed neurologic signs.

To determine DNA synthesis rates, 5 μCi in 91 nmoles [^{14}C]thymidine (New England Nuclear

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† The diets contained casein, vitamin-free test, GBI (180 g/kg), corn starch (100 g/kg), sucrose (590 g/kg), corn oil (80 g/kg), salt mix, William–Briggs Modified (40 g/kg), vitamin mixture, with or without thiamine (catalog No. 40060, 10 g/kg).

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Corp., Boston, Mass.) was injected i.p. when neurological signs were noted. Rats were then sacrificed by decapitation at appropriate time intervals, the organs removed as rapidly as possible, and frozen on dry ice. "Reversed" rats received 500 μg thiamine HCl (Sigma Chemical Co., St. Louis, Mo.) at 24-hr intervals and were sacrificed at 24 or 48 hr after the initial injection. Reversed rats were fed *ad lib.* or, as indicated, were fasted by restricting food intake to 1 g/day along with their pair-fed controls. The latter was done since thiamine-deficient rats are anorexic and may, in addition, absorb or utilize food in an abnormal manner [3] (see Fig. 1). Thus, food was withheld from some TD rats and their pair-fed controls after thiamine repletion, in order to determine if the rebound in DNA synthesis seen in reversed TD rats was caused by thiamine repletion or by the increased food intake which follows it. TD rats normally consume 1–2 g/day immediately prior to appearance of neurological signs.

Thiamine-deficient rats are also hypothermic in the severe stages of dietary thiamine deficiency [10]. The body temperature of symptomatic TD rats declines to a mean of 32.2°; thus, experiments were done on ten sets of rats in which the thiamine-deficient rat was warmed to normal body temperature for 2 hr prior to the injection of the labeled thymidine and for the 3 subsequent hr before sacrifice. Tissue DNA synthesis was also determined in the normothermic TD rats.

Extraction of organs

To extract total RNA and DNA, the frozen organs were placed in 20% perchloric acid at 4° (approximately 1 ml/200 mg of tissue). The organs were then homogenized in a glass tissue grinder and the DNA and RNA extracted by the method of Castles *et al.* [11].

Analytical techniques

DNA was assayed by the Burton [12] modification of the dephenylamine method, RNA by the orcinol reaction [13], and protein by the Hartree [14] modification of the Lowry technique.

The identity of the ^{14}C-label incorporated into organ DNA was determined by descending paper chromatography [Whatman No. 3, 2-propanol–concentrated HCl–H_2O (65:17.2:17.8)] of organ DNA hydrosylates. The DNA extracts from the 3-hr injections were pooled for each organ and neutralized with $KClO_4$. The supernatant was concentrated by freeze drying (Virtis, Gardiner, N.Y.), the residue was suspended in 6 N HCl for 3 hr at 90°, the hydrosylate was again lyophilized, and the residue was dissolved in 0.5 ml H_2O, of which 10 μl was spotted for chromatography. The resulting four spots, adenine, guanine, cytosine and thymine (R_f values of 0.28, origin to 0.19, 0.46, 0.78, respectively), were cut out and their radioactivity was determined.

Radioactivity of the samples was determined by adding 1 ml of sample to 15 ml fluor [toluene–Triton X-100 (2:1), PPO (3.0 g/liter), POPOP (0.13 g/liter)] followed by counting in a Packard Tricarb scintillation counter. [^{14}C]thymidine incorporation into DNA was expressed as dis./min/mg of DNA. Statistical significance between thiamine-deficient rats and controls as well as between pair-fed and *ad lib.* controls was determined by the Student's paired *t*-test.

RESULTS

Symptomatic stage

Growth rate. Figure 1 illustrates body growth rates in the thiamine-deficient (TD), pair-fed control (PFC), and *ad lib.* fed control (C) rats used to evaluate this model of diet-induced thiamine deficiency (N = 12). Body weight in the *ad lib.* controls increased steadily throughout the dietary regimen while the weight of PFC rats leveled out at about 120 g after 2 weeks of pair-feeding. Body weight of TD rats declined steadily after 12 days to slightly over 70 g during the symptomatic stage [37 ± 1 days (mean ± S.E.) from the start of the diet]. These data indicate, as has been demonstrated before [3], that despite assiduous pair-feeding, TD rats assimilated less of the administered food and/or did not utilize it comparably to the PFC animals.

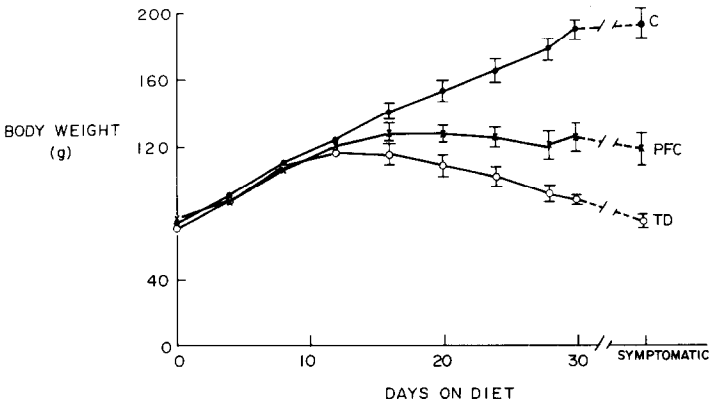

Fig. 1. Body weight curve for thiamine-deficient and control rats. TD refers to thiamine-deficient, PFC to pair-fed control, and C to *ad lib.* control rats (N = 12). The points represents the mean ± standard error of twelve rats. Each point was statistically analyzed by the *t*-test. TD rats were statistically lower than both control groups ($P < 0.05$) after 12 days. PFC rats were lower in weight than *ad lib.* controls also after 12 days ($P < 0.05$).

Table 1. Organ weights and protein levels in thiamine-deficient symptomatic and thiamine-injected ("reversed") rats

Organ		Organ weights (g)			(mg Protein/organ)			(mg Protein/g tissue)		
		TD	PFC	C	TD	PFC	C	TD	PFC	C
Heart										
Symptomatic*		0.358 ± 0.02†	0.423 ± 0.03	0.752 ± 0.06	49.8 ± 7.3†	68.1 ± 11	113.7 ± 16.8	138.4 ± 17.3*	160.1 ± 19.9	151.8 ± 20.7
Reversed‡										
Non-	(24 hr)	0.361 ± 0.015	0.370 ± 0.024	0.749 ± 0.037	66.8 ± 11.2	67.1 ± 4.9	128 ± 12.6	183.6 ± 28.7	184.5 ± 19.7	184.4 ± 15.2
fasted	(48 hr)	0.377 ± 0.036†	0.440 ± 0.03	0.796 ± 0.043	59.6 ± 10.4	67.9 ± 7.7	127 ± 13.6	159.6 ± 26.7	153.9 ± 12.8	159.9 ± 15.9
Fasted	(48 hr)	0.349 ± 0.025†	0.385 ± 0.02	0.831 ± 0.04	60.3 ± 7.6†	69.6 ± 9.6	162.9 ± 29.3	179.1 ± 31	184.8 ± 28.2	197.2 ± 37.6
Kidney										
Symptomatic		1.12 ± 0.09	1.07 ± 0.12	1.86 ± 0.16	158.3 ± 23.4	151.6 ± 13.8	228.1 ± 39.4	144.4 ± 22.6	145.9 ± 16.2	136.5 ± 39.7
Reversed										
Non-	(24 hr)	1.09 ± 0.39	0.867 ± 0.054	1.72 ± 0.094	180.5 ± 20.4	165.9 ± 19.3	253.5 ± 36.9	167.5 ± 21	196.6 ± 28.9	147.2 ± 18.1
fasted	(48 hr)	1.15 ± 0.07	1.10 ± 0.049	2.04 ± 0.2	183.5 ± 21.5	183.4 ± 22.8	329 ± 43.6	161.7 ± 22.7	181.6 ± 21.7	164.7 ± 22
Fasted	(48 hr)	0.994 ± 0.09	0.936 ± 0.053	2.10 ± 0.2	161.8 ± 12.5	166.4 ± 20.8	278.4 ± 70.7	116.9 ± 19.6	178.9 ± 21.6	169.5 ± 18.6
Pancreas										
Symptomatic		0.380 ± 0.04†	0.497 ± 0.05	0.775 ± 0.14	64.2 ± 10.9†	84.9 ± 10.4	124 ± 18.4	169.7 ± 20.6	172.3 ± 16.5	164.6 ± 21.9
Reversed										
Non-	(24 hr)	0.369 ± 0.032	0.358 ± 0.027	0.703 ± 0.077	51.2 ± 5.0†	66.2 ± 14.7	144.8 ± 18.5	141.3 ± 14.4	184.2 ± 37.2	214.6 ± 21.4
fasted	(48 hr)	0.504 ± 0.047	0.516 ± 0.094	0.953 ± 0.097	64.9 ± 10.4	83.0 ± 22.6	147.2 ± 16.8	127.3 ± 11.2†	156.7 ± 22.9	157 ± 17.2
Fasted	(48 hr)	0.418 ± 0.073†	0.444 ± 0.038	0.928 ± 0.079	55.6 ± 9.6†	79.4 ± 16.9	185.5 ± 35	139.9 ± 25†	179.7 ± 38.1	205.5 ± 41.4
Liver										
Symptomatic		2.30 ± 0.23†	3.50 ± 0.29	7.23 ± 0.44	485.6 ± 67.9†	624.3 ± 58.4	1,042.9 ± 152.5	208.4 ± 23.5	183.2 ± 24.8	146.5 ± 24.8
Reversed										
Non-	(24 hr)	3.95 ± 0.32†	3.31 ± 0.44	6.37 ± 0.74	516.9 ± 117.6	678.4 ± 127.3	1,188.1 ± 159.4	193.5 ± 94.6	208.9 ± 44.2	188.7 ± 18.1
fasted	(48 hr)	4.60 ± 0.62	4.76 ± 0.75	7.77 ± 0.46	886.9 ± 162.9	796.7 ± 174.2	1,788.8 ± 514	195.6 ± 17.3	167.5 ± 19.1	224.3 ± 52.2
Fasted	(48 hr)	1.93 ± 0.19	2.3 ± 0.25	7.47 ± 0.53	369.8 ± 53.9†	509.7 ± 78.4	1,518.6 ± 227	207.8 ± 21	228.1 ± 25.9	229.6 ± 36.3

* Symptomatic refers to the TD rats that show overt neurological dysfunction.

† Signifies that TD is significantly different from PFC at the $P < 0.05$ level ($N = 22$ for symptomatic and $N = 12$ for each reversed time).

‡ Reversed indicates that symptomatic rats were injected i.p. with 500 μg thiamine at 24-hr intervals. Neurologic signs disappeared within 6 hr after thiamine injection. Times indicate the interval between initial thiamine repletion and the time at which 5 μCi (91 pmoles in 50 μl) of [^{14}C]thymidine was injected i.p. Rats were sacrificed 3 hr after injection of the isotope. Non-fasted TD rats received a thiamine-deficient diet *ad lib.*, while fasted TD rats and their pair-fed controls were restricted to 1 g/24 hr for 2 days after initial thiamine repletion.

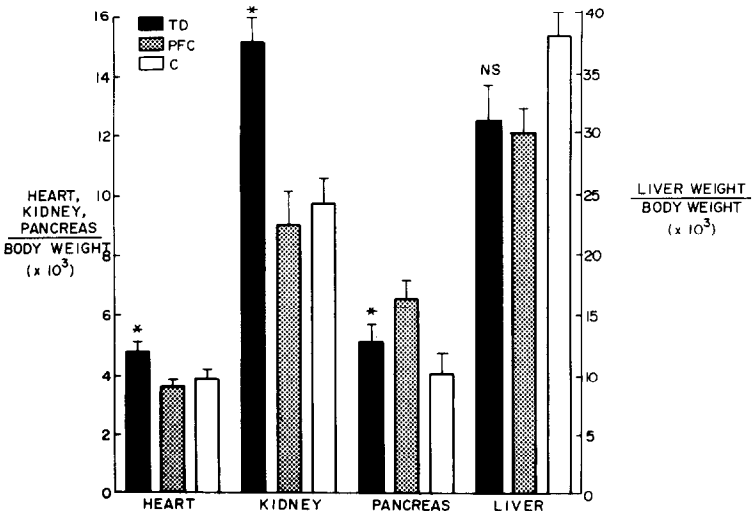

Fig. 2. Effect of thiamine deficiency on the organ body weight ratio. The left vertical axis represents the ratio of heart, kidney and pancreas wet weight to body weight and the right vertical axis indicates the same ratio for liver. Asterisks indicate a significant difference ($P < 0.05$) between symptomatic TD and PFC values ($N = 22$). Vertical lines represents standard errors of 22 animals in each group and NS indicates no significant difference between TD and PFC rats.

Organ weight and composition. The effect of dietary thiamine deficiency on organ weight is shown in Table 1. It can be seen in the table that net weights of the heart, pancreas and liver were significantly reduced ($P < 0.05$) in symptomatic TD rats ($N = 22$) as compared to their pair-fed controls, while kidney weight was similar. As compared to *ad lib.* fed control values, all organ weights in TD and PFC rats were substantially reduced ($P < 0.05$). When organ weights are expressed as a fraction of total body weight (Fig. 2), heart and kidney weights in TD rats were significantly higher ($P < 0.05$) than in PFC animals, while the weight of the pancreas was lower. Liver weight, as per cent of body weight, was the same in TD and PFC rats, and both were lower than the *ad lib.* control values. These results reflect the substantial body weight loss of the TD animals and indicate that heart and kidney lost less substance as compared to total body weight, that liver mass declined at an equal rate, and that pancreatic weight fell preferentially.

The protein content and levels in various organs are shown in Table 1. Total protein in symptomatic TD heart, pancreas and liver was reduced from PFC values ($P < 0.05$), while kidney protein content remained unchanged. The protein content of all organs in *ad lib.* fed controls was higher ($P < 0.05$) than that of both TD and PFC rats. When protein is expressed as mg/g of tissue, only the TD heart level is reduced significantly ($P < 0.05$) from PFC values and even this decrease is small. These data indicate that, despite a significant decrease in total body and various organ weights with diet-induced thiamine deficiency, the protein concentration in the viscera of TD rats remained essentially normal.

Total DNA content in various organs of symptomatic TD rats was comparable to that of PFC animals, except for the liver, which showed a 16 per cent reduction (Table 2). TD and PFC DNA content in all organs ($N = 22$) except pancreas was lower than that for the *ad lib.* controls ($P < 0.05$). Heart, pancreas and liver DNA levels, expressed as mg DNA/g of tissue, were significantly increased in symptomatic TD rats ($P < 0.05$), while kidney DNA was not altered. *Ad lib.* controls showed the lowest DNA concentration for all organs. Expressed per mg of tissue protein, only the TD heart and pancreas were higher than pair-fed controls ($P < 0.05$). In summary, the increased DNA concentrations in TD organs shown in Table 2 reflect a selectively greater loss of cytoplasmic mass (non-protein material) than of DNA in TD viscera. The decrease in DNA content of TD liver, however, also implies some reduction in the overall number of cell nuclei and therefore of cells in this organ.

Total RNA content and concentration in symptomatic TD rats and appropriate controls ($N = 22$) are shown in Table 3. Total RNA content in TD heart and liver was significantly below PFC values ($P < 0.05$), and remained unchanged in kidney and pancreas. In terms of RNA concentration (μg RNA/g of tissue), only TD liver exhibited a decreased RNA level ($P < 0.05$), as compared to PFC rats. Comparable data are noted when organ RNA is expressed per g of tissue protein. Thus, the main finding was a 24 per cent decrease in liver RNA concentration in TD rats.

DNA synthesis. Synthesis rates of DNA were determined by [^{14}C]thymidine incorporation into organ DNA. Incorporation was found to be linear in all four organs, up to 3 hr. The DNA synthesis rates were similarly depressed as compared to PFC at all time periods studied (30, 90, 120 and 180 min after [^{14}C]thymidine injection). Also, with the exception of the pancreas, PFC values were less than *ad lib.* control rates ($P < 0.05$) (by 68, 62 and 53 per cent for heart, kidney and liver respectively). Figure 3 (the bars labeled SYMP) indicates the marked depression in DNA synthesis in symptomatic TD rats ($N = 12$) at 3 hr after [^{14}C]thymidine injection. Synthesis rates in heart, kidney, pancreas and liver were reduced to 36, 52, 32 and 64 per cent of PFC values ($P < 0.05$). Analysis of labeled DNA, extracted from organs of

Table 2. Organ DNA levels in thiamine-deficient symptomatic and thiamine-injected ("reversed") rats*

Organ		Total DNA/organ (mg)			(mg DNA/g tissue)			(μg DNA/mg protein)		
		TD	PFC	C	TD	PFC	C	TD	PFC	C
Heart										
Symptomatic		0.915 ± 0.05	0.955 ± 0.07	1.07 ± 0.07	2.58 ± 0.27†	2.27 ± 0.27	1.43 ± 0.19	20.1 ± 3.9†	15.3 ± 2.6	10.1 ± 0.1
Reversed										
Non-	(24 hr)	0.979 ± 0.36	0.887 ± 0.13	1.08 ± 0.12	2.65 ± 0.89	2.47 ± 0.42	1.46 ± 0.42	14.2 ± 3.7	13.4 ± 2.2	8.31 ± 1.02
fasted	(48 hr)	0.823 ± 0.16	1.05 ± 0.14	1.29 ± 0.17	2.15 ± 0.27	2.37 ± 0.23	1.62 ± 0.17	14.9 ± 3.1	15.5 ± 1.1	10.5 ± 1.4
Fasted	(48 hr)	0.886 ± 0.14	0.865 ± 0.15	1.23 ± 0.1	2.57 ± 0.41	2.28 ± 0.42	1.50 ± 0.17	16.0 ± 3.6	14.3 ± 4.2	8.47 ± 1.78
Kidney										
Symptomatic		6.11 ± 0.35	6.35 ± 0.45	7.19 ± 1.0	5.50 ± 0.50	6.05 ± 0.68	3.93 ± 0.35	37.4 ± 6.5	42.4 ± 5.9	35.6 ± 8.6
Reversed										
Non-	(24 hr)	6.30 ± 0.37	6.14 ± 0.41	7.02 ± 0.39	5.82 ± 0.37†	7.10 ± 0.38	4.63 ± 0.75	36.7 ± 5.4	39.6 ± 7.2	28.6 ± 3.4
fasted	(48 hr)	5.91 ± 0.56	7.15 ± 1.5	8.44 ± 1.38	5.16 ± 0.46	6.96 ± 1.2	4.08 ± 0.34	35.2 ± 6.0	35.5 ± 5.6	26.8 ± 4.6
Fasted	(48 hr)	6.76 ± 0.53	6.87 ± 0.57	9.03 ± 0.91	6.97 ± 0.74	7.40 ± 0.71	4.34 ± 0.36	43.6 ± 7.5	43.7 ± 7.1	26.6 ± 3.4
Pancreas										
Symptomatic		2.29 ± 0.19	2.41 ± 0.48	2.78 ± 0.50	6.23 ± 0.63†	4.02 ± 0.79	3.53 ± 0.47	36.4 ± 4.6†	29.9 ± 5.6	25.8 ± 6.5
Reversed										
Non-	(24 hr)	2.44 ± 0.21	2.77 ± 0.30	3.39 ± 0.42	6.67 ± 0.87	7.87 ± 0.96	5.06 ± 0.51	49.1 ± 7.8	47.2 ± 9.2	23.9 ± 2.0
fasted	(48 hr)	2.90 ± 0.42	3.29 ± 0.49	3.85 ± 0.50	5.77 ± 0.53	6.58 ± 0.63	4.01 ± 0.40	46.1 ± 4.7	39.6 ± 5.9	26.8 ± 0.36
Fasted	(48 hr)	2.85 ± 0.27	3.19 ± 0.21	4.14 ± 0.53	7.14 ± 0.68	7.32 ± 0.68	4.46 ± 0.39	56.9 ± 11.7	48.2 ± 11.4	25.0 ± 5.6
Liver										
Symptomatic		9.29 ± 1.41†	11.0 ± 1.7	14.6 ± 1.6	4.08 ± 0.60†	3.15 ± 0.45	2.05 ± 0.25	18.8 ± 2.8	17.6 ± 2.8	15.4 ± 2.9
Reversed										
Non-	(24 hr)	8.89 ± 1.21†	12.3 ± 1.4	17.0 ± 2.0	2.22 ± 0.29†	3.17 ± 0.32	3.11 ± 0.23	19.5 ± 4.8	21.4 ± 5.5	17.0 ± 2.0
fasted	(48 hr)	9.84 ± 1.7†	12.7 ± 1.8	18.6 ± 2.4	2.28 ± 0.32†	2.96 ± 0.66	2.38 ± 0.23	12.4 ± 2.0†	18.1 ± 3.5	12.9 ± 0.9
Fasted	(48 hr)	8.70 ± 1.7†	12.3 ± 1.6	19.5 ± 1.7	4.55 ± 0.76	5.43 ± 0.55	2.65 ± 0.31	24.5 ± 4.4	25.8 ± 4.1	13.5 ± 1.9

* See legend to Table 1 for details of experiments.

† Signifies that TD is significantly different from PFC at the $P < 0.05$ level ($N = 22$ for symptomatic and $N = 12$ for each reversed time).

Table 3. Organ RNA levels in thiamine-deficient symptomatic and thiamine-injected ("reversed") rats*

Organ		Total RNA/organ (mg)			(mg RNA/g tissue)			(μg RNA/mg protein)		
		TD	PFC	C	TD	PFC	C	TD	PFC	C
Heart										
Symptomatic		0.240 ± 0.06†	0.270 ± 0.07	0.410 ± 0.07	0.701 ± 0.20	0.642 ± 0.16	0.560 ± 0.10	5.17 ± 1.85	4.59 ± 1.74	3.87 ± 0.86
Reversed										
Non-	(24 hr)	0.318 ± 0.041	0.351 ± 0.05	0.589 ± 0.08	0.881 ± 0.126	0.947 ± 0.104	0.786 ± 0.097	5.94 ± 2.2	5.33 ± 0.82	4.49 ± 0.59
fasted	(48 hr)	0.338 ± 0.05	0.317 ± 0.028	0.637 ± 0.05	0.890 ± 0.108†	0.749 ± 0.075	0.804 ± 0.079	6.54 ± 1.39	5.02 ± 0.75	4.99 ± 0.63
Fasted	(48 hr)	0.280 ± 0.044	0.286 ± 0.034	0.672 ± 0.106	0.794 ± 0.085	0.884 ± 0.206	0.798 ± 0.097	5.27 ± 1.63	4.55 ± 1.25	4.56 ± 1.06
Kidney										
Symptomatic		1.50 ± 0.48	1.39 ± 0.37	2.95 ± 0.62	1.30 ± 0.34	1.33 ± 0.32	1.63 ± 0.25	9.30 ± 4.0	8.72 ± 2.73	15.2 ± 5.4
Reversed										
Non-	(24 hr)	1.33 ± 0.11	1.10 ± 0.15	2.71 ± 0.25	1.23 ± 0.10	1.27 ± 0.15	1.78 ± 0.27	7.84 ± 1.13	7.04 ± 1.34	11.0 ± 1.41
fasted	(48 hr)	1.85 ± 0.23	1.80 ± 0.42	3.76 ± 0.51	1.59 ± 0.13	1.76 ± 0.34	1.79 ± 0.11	10.1 ± 1.5	8.76 ± 1.16	11.8 ± 2.2
Fasted	(48 hr)	1.52 ± 0.20	1.55 ± 0.18	4.36 ± 0.72	1.52 ± 0.10	1.64 ± 0.13	2.01 ± 0.21	9.59 ± 1.32	11.0 ± 1.89	15.7 ± 5.1
Pancreas										
Symptomatic		1.38 ± 0.48	1.54 ± 0.29	2.77 ± 0.57	3.41 ± 0.77	3.15 ± 0.54	3.63 ± 0.87	23.0 ± 8.6	18.2 ± 3.6	23.8 ± 5.7
Reversed										
Non-	(24 hr)	1.46 ± 0.37	1.42 ± 0.37	3.15 ± 0.57	3.96 ± 1.07	3.91 ± 0.93	4.95 ± 0.88	28.5 ± 7.5	23.9 ± 7.1	23.0 ± 2.3
fasted	(48 hr)	2.96 ± 0.36	2.37 ± 0.39	6.20 ± 0.80	5.89 ± 0.63	5.38 ± 1.02	6.60 ± 0.88	51.0 ± 4.7†	38.6 ± 10.2	45.1 ± 7.8
Fasted	(48 hr)	3.37 ± 1.44	2.57 ± 0.21	5.73 ± 0.50	6.39 ± 0.62	5.36 ± 0.92	6.33 ± 0.80	52.3 ± 10.7†	38.1 ± 9.2	34.2 ± 6.5
Liver										
Symptomatic		4.63 ± 1.42	9.51 ± 2.4	23.2 ± 4.0	2.05 ± 0.59†	2.68 ± 0.55	3.21 ± 0.47	11.6 ± 4.3†	15.4 ± 3.7	25.5 ± 6.9
Reversed										
Non-	(24 hr)	6.26 ± 2.49	6.05 ± 1.29	26.1 ± 2.7	1.52 ± 0.51	1.83 ± 0.37	4.27 ± 0.65	13.6 ± 6.1	10.7 ± 3.7	22.8 ± 2.7
fasted	(48 hr)	11.03 ± 3.4	9.48 ± 2.64	27.7 ± 3.2	2.23 ± 0.41	2.03 ± 0.37	3.52 ± 0.20	11.4 ± 2.6	12.1 ± 2.6	16.9 ± 2.3
Fasted	(48 hr)	3.64 ± 1.19	4.58 ± 1.3	27.5 ± 3.0	1.77 ± 0.46	1.85 ± 0.41	3.71 ± 0.28	10.1 ± 3.3	8.82 ± 2.18	19.9 ± 4.6

* See legend to Table 1 for details of experiments.

† Signifies that TD is significantly different from PFC at the $P < 0.05$ level ($N = 22$ for sympromatic and $N = 12$ for each reversed time).

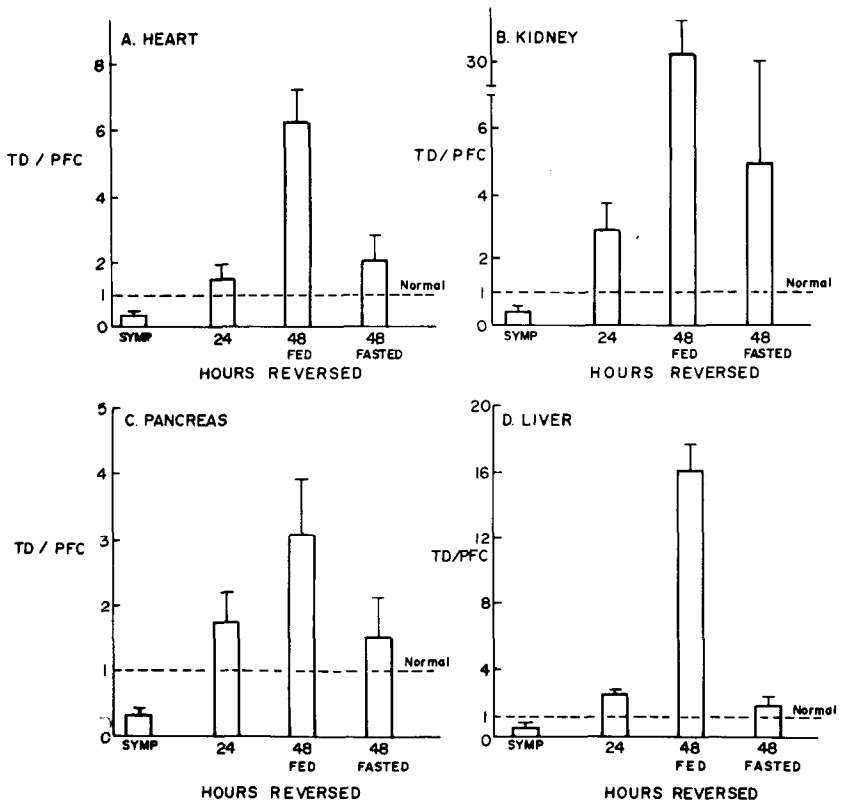

Fig. 3. Effect of thiamine repletion of thiamine-deficient rats on organ DNA synthesis. The vertical axis (TD/PFC) represents the ratio of DNA labeling of thiamine-deficient (TD) rats and pair-fed controls (PFC) 3 hr after an i.p. injection of 5 μCi (91 pmoles) of [^{14}C]thymidine. "Normal" values (TD = PFC) are indicated by the horizontal dotted line. "SYMP" refers to the labeling ratio when the TD rats show neurological dysfunction (N = 12). "Hours reversed" represents the time from the initial i.p. injection of 500 μg thiamine (injected at 24-hr intervals) until injection of [^{14}C]thymidine. Neurologic signs were reversed within 6 hr of thiamine injection. FED indicates that the reversed TD rats were allowed to consume thiamine-deficient diet *ad lib.* for 24 or 48 hr prior to sacrifice while FASTED means that TD and PFC food was restricted to 1 g/24 hr. Vertical lines indicate standard errors of twelve rats in each group.

TD, PFC and C rats, showed that virtually all of the isotope was in the DNA thymine.

To ascertain that the hypothermia of thiamine deficiency was not the cause of decreased DNA labeling in TD organs, DNA synthesis rates were determined in ten sets of rats in which symptomatic TD animals were warmed to normal body temperature. No change ($P < 0.05$) in the labeling pattern from hypothermic rats was observed, eliminating non-specific hypothermia as the mechanism of the impaired DNA synthesis in TD animals.

Reversal of thiamine deficiency

DNA synthesis. As previously mentioned, the neurologic deficit encountered in TD rats can be completely reversed within 6 hr by a single i.p. injection of 500 μg thiamine. Since thiamine-deficient rats given thiamine increase their food consumption rapidly, some (N = 12 for each "reversed" time interval) of the "reversed" animals were given access to food freely and others (N = 12) were restricted to 1 g of food/day. This design was used to attempt to separate the effect of thiamine restoration alone from that of increased food intake. Figure 3 depicts [^{14}C]thymidine incorporation into organ DNA during and after reversal of neurologic dysfunction of thiamine deficiency (N = 12). In the heart, DNA labeling rose from 36 per cent of PFC values during severe thiamine deficiency to 142 and 622 per cent of PFC data 24 and 48 hr, respectively, after thiamine injection. When fasting accompanied the 48-hr reversal, the increase in DNA labeling was held to twice the PFC level ($P < 0.05$). Incorporation rates into kidney DNA rose from 52 per cent of PFC values to 287 per cent at 24 hr and to 3200 per cent at 48 hr after reversal. With fasting, thiamine administration caused DNA synthesis to be elevated to 491 per cent of PFC values at 48 hr ($P < 0.05$). The 68 per cent reduction in ^{14}C-labelling of pancreatic DNA in TD rats was rapidly reversed with thiamine, increasing to 172 and 307 per cent of PFC values at 24 and 48 hr. Again, concomitant fasting reduced the 48-hr increase to 152 per cent of PFC results ($P < 0.05$). During neurologic dysfunction, liver [^{14}C]thymidine incorporation in TD rats was reduced to 64 per cent of PFC animals. Reversal over 24 and 48 hr increased DNA labeling to 244 and 1600 per cent of control. However, fasting held the latter increase to only 171 per cent ($P < 0.05$). Although not illustrated in this paper, a series (N = 7) of experiments was done in which rats

Table 4. Incorporation of [^{14}C]thymidine into organ DNA during the symptomatic stage or at 48 hr after reversal of the neurologic signs*

Organ	TD reversed (fasted)† / TD (symptomatic)	TD reversed (fasted)‡ / TD reversed (*ad lib.*)	PFC reversed (fasted) / PFC (symptomatic)	PFC reversed (fasted) / PFC (*ad lib.*)
Heart	2.19§,‖	0.163	0.464	0.480
Kidney	4.70	0.122	0.415	0.357
Pancreas	1.78	0.179	0.365	0.312
Liver	1.19	0.055	0.513	0.540

* Symptomatic stage refers to presence of neurologic signs. Reversal refers to elimination of neurologic signs by the administration of thiamine.

† Fasted refers to an experimental design where food intake by TD and PFC rats was reduced to 1 g/day.

‡ *Ad lib.* refers to unrestricted food intake.

§ Statistical evaluation of data is given in the text.

‖ Numerators and denominators of the fractions were derived from means where N = 12 for (symptomatic), N = 12 for reversed (*ad lib.*) and N = 12 for reversed (fasted).

were injected with [^{14}C]thymidine over a 1.5-hr time interval at the end of 48 hr of thiamine repletion. Reversal of DNA synthesis inhibition was comparable to that seen with the 3-hr injections (Fig. 3). Thus, in all organs, a 48-hr reversal of thiamine deficiency (accompanied by unrestricted food intake) increased DNA labeling (at 1.5- and 3-hr injection times) to values considerably above pair-fed and even those of *ad lib.* controls ($P < 0.05$). However, when reversal was accompanied by restricted food intake, the increased labeling of DNA in reversed TD rats was blunted. During 48 hr of reversal and starvation, TD DNA labeling rates increased by 219, 491, 152 and 171 per cent of TD symptomatic heart, kidney, pancreas and liver respectively (Table 4). The starvation caused large variation in data and this TD increase was significantly higher than symptomatic TD values only in the kidney ($P < 0.05$). This increase was significantly lower than when food was freely supplied. The continued increased food restriction in PFC rats reduced DNA labeling in heart, kidney, pancreas and liver by 54, 59, 64 and 49 per cent, respectively (Table 4), as compared to PFC values obtained during symptomatic thiamine deficiency ($P < 0.05$). As a result of the increase in TD labeling and a fall in PFC DNA formation, the TD/PFC DNA synthesis ratio rose significantly after reversal even with fasting ($P < 0.05$).

Organ weight and composition. Tables 1–3 summarize the effects of thiamine repletion of TD rats on organ weight and protein concentration as well as on DNA and RNA levels in the four organs (N = 12 for each "reversed" time). As indicated in Table 1, pancreas and liver weights returned to PFC values after 48 hr of reversal ($P < 0.05$), if the rats were fed freely. Organ weight as a function of body weight remained normal for the liver after a 48-hr reversal, while the fraction for the TD pancreas returned to its PFC value. If food was restricted to 1 g/day after reversal, the weight of the pancreas remained below ($P < 0.05$) the PFC value, the TD liver weight remained constant, and PFC liver weight declined. Kidney weight was unaffected by thiamine deficiency, while TD heart weight remained below the PFC value ($P < 0.05$) even after 48 hr of reversal with food. The organ/body weight ratio for the kidney and heart both returned to control values after this 48-hr reversal. Total protein/organ, which was significantly reduced in symptomatic TD heart, pancreas and liver, increased to PFC values 48 hr after thiamine, if normal feeding was allowed. Fasting blocked this increase in total protein in all three organs and, in fact, induced a further decrease in protein content in TD pancreas and liver. When protein was expressed per g tissue (Table 1), a significant decrease ($P < 0.05$) was seen only in symptomatic TD heart and this effect was reversed with thiamine with or without normal food intake. In general, the reversal of thiamine deficiency with provision of adequate food increased the organ weight and protein content of most organs and this effect was blunted or abolished by food deprivation in at least some tissues.

Organ DNA values are shown in Table 2. Total DNA was only decreased in TD liver and this was not altered by the administration of thiamine over 48 hr. DNA levels, calculated per g of tissue, were higher in symptomatic TD heart, pancreas and liver. A 48-hr reversal, irrespective of food intake, decreased the DNA level in heart and pancreas to PFC values ($P < 0.05$). The liver DNA concentration in reversed TD rats actually fell below PFC values and this was statistically significant in the fed group ($P < 0.05$). In general, fasting plus thiamine injection in TD rats and fasting alone in PFC rats increased the DNA concentration in the kidney, pancreas and liver. DNA levels, expressed per g of tissue protein, were also higher in symptomatic TD heart and pancreas, and thiamine treatment, even with fasting, reversed this effect. These data clearly show that: (1) the decreased DNA content of TD liver is not rapidly reversible with thiamine repletion, and (2) the increased DNA concentration of TD organs generally responds readily to thiamine.

Organ RNA values are shown in Table 3. Total organ RNA was significantly decreased only in TD liver and heart (slightly in the latter). Both values returned to normal after reversal of neurologic signs. Fasting during reversal dramatically decreased liver RNA in both TD and PFC rats. RNA concentration in TD liver (as compared to PFC data) was significantly decreased and this was reversed after thiamine administration, with or without fasting. The apparent response with fasting, however, was due in part to a greater fall in PFC RNA concentration. Group C tissue levels for protein, DNA and RNA did not differ significantly in symptomatic and reversed experiments.

DISCUSSION

The first finding in this study is the demonstration of a major depression of DNA synthesis *in vivo* in key viscera (heart, pancreas, kidney and liver) of thiamine-deficient rats. Although only the 3-hr injection period is illustrated, DNA synthesis was found to be depressed at all time points between 30 and 180 min, during which time [^{14}C]thymidine incorporation into DNA was linear. This is consistent with our recent report of decreased DNA formation in the brains of such animals [9]. Incorporation of labeled thymidine into organ DNA was employed as an index of DNA synthesis. This approach was validated by: (1) the use of methods which rigorously extract and purify DNA obtained from tissues, (2) the demonstration that virtually all the label in extracted DNA existed in the thymine moiety, (3) the documentation that impaired DNA labeling in thiamine-deficient tissues was not a non-specific effect of hypothermia which accompanies the experimental diet-induced thiamine deficiency state [10], and (4) the measurement of DNA formation at time intervals after injection of the isotope (30–180 min), which is likely to obviate any transient decrease in uptake of the label by thiamine-deprived organs. The use of [^{14}C]thymidine incorporation into tissue DNA as a valid indicator of DNA synthesis assumes that there is no increase in endogenous thymidine in thiamine-deficient viscera which might dilute the administered isotope and cause an apparent decrease in labeling of the DNA. This subject was not specifically assessed in this study, but prior investigations in our unit showed no increase in cerebral endogenous thymidine in thiamine deficiency [9].

Despite a substantial (40–70 per cent) depression of DNA synthesis with severe thiamine deficiency in all the tissues studied, total DNA content of heart, pancreas and kidney remained unaltered (as compared to pair-fed thiamine-repleted rats), and liver DNA content declined by only 16 per cent. Furthermore, on administration of thiamine and reversal of the neurologic signs, there was a brisk and striking (16-fold for liver) increase in DNA synthesis in all the organs, but tissue DNA content did not increase. This suggests that the thiamine deficiency state depresses, and its removal increases, DNA turnover of only a small DNA pool. In a previous report concerning brain DNA synthesis in thiamine deficiency, a similar interpretation was reached and it was suggested that thiamine deficiency may impair primarily the DNA repair process [9]. The same possibility exists as regards the organs studied in this report, but this hypothesis clearly requires experimental verification. An alternate interpretation of altered DNA synthesis and relatively fixed DNA tissue content would imply a proportional but opposite change in DNA utilization. There are no data available at present to assess this possibility.

In order to assess the specificity and reversibility of impaired DNA synthesis in thiamine deficiency, the deficient animals were given thiamine, resulting in rapid resolution of neurologic signs, and DNA synthesis was again examined. There was a rapid (within 24 hr) and substantial increase in DNA synthesis in all organs, and this reached supranormal levels in 48 hr in all instances. Unfortunately this technique, which is generally used to assess the specificity of thiamine deficiency *per se*, does not rule out a concomitant nutritional effect. Diet-induced thiamine deficiency in experimental animals induces anorexia and subsequent weight loss. As can be seen from Fig. 1 and other studies [3], this effect cannot be fully compensated for by meticulous pair-feeding of controls. This implies that despite provision of comparable dietary intake, the thiamine-deficient rats must either assimilate food poorly or utilize it differently. The latter mechanism has been documented in a recent study [15]. DNA synthesis can be altered by dietary changes and falls with food and/or protein deprivation [16, 17]. Thus, abnormal DNA synthesis in symptomatic thiamine-deficient rats could be caused either by thiamine deficiency *per se*, a secondary abnormality of nutrition, or both. After reversal of neurologic signs with thiamine, there is a rapid improvement in appetite and food intake. Thus, even in 24 hr, an improvement in DNA synthesis may again be due to provision of thiamine, improvement in nutrition, or both. [DNA synthesis is not affected in normal (thiamine replete) rats after i.p. injection of 500 μg thiamine for 48 hr.] In an attempt to separate the possible effects of thiamine deficiency alone from its nutritional effects, a series of experiments was carried out wherein thiamine-deficient rats were injected with thiamine, and food was severely restricted in both them and the PFC rats during the reversal period. From these data it is evident that food deprivation depressed tissue DNA synthesis in both TD and PFC rats but it also appears that increase of DNA synthesis by the administration of thiamine is only partly blocked by starvation. This depression of DNA labeling by starvation is similar to that previously reported for liver and kidney during fasting and protein deficiency [16, 17]. Sterling *et al.* [18] have also recently shown that the proliferative response seen after partial hepatectomy can be impaired by starvation. A similar mechanism may be occurring in the thiamine-deficiency system. While the fasting experiment does not clearly separate a direct action of thiamine from a secondary effect resulting from poor food assimilation or utilization, it does suggest that thiamine *per se* may have a role in the synthesis of some pool(s) of DNA in various tissues.

The second relevant set of observations derived from this study relates to the effect of the thiamine-deficiency state on organ composition. As stated earlier, the thiamine-deficient rats and the pair-fed controls (to a lesser extent) lost weight briskly during the study. This weight loss in TD rats was matched by a proportional decrease in liver mass and a greater fall in pancreatic mass while the heart and kidneys lost less weight. These observations are in agreement with less extensive data reported previously by Schenker *et al.* [19]. The cause(s) of organ weight loss in thiamine deficiency varied with the tissue. In the liver, there was a statistically significant decrease in total DNA content, hence probably a reduction in the total number of liver cells. Yet the total hepatic DNA content decreased by only 16 per cent as compared to a reduction in total protein of 22, organ weight of 34, and RNA of 51 per cent, respectively, in relation to PFC values. Thus, the protein concentration in the liver did not change significantly but

the RNA concentration fell ($P < 0.05$) and the DNA concentration rose slightly ($P < 0.05$) when expressed per g of tissue weight (Tables 1–3). These data suggest that, in addition to a decrease in cell number, TD rats exhibited an even greater reduction of individual cell substance(s). This loss in liver cell weight was not accounted for by a decrease in total protein or cell water and will require future elucidation. In sharp contrast to the liver, thiamine-deficient kidney did not exhibit any difference in organ weight or DNA and RNA content from pair-fed data. The heart and pancreas showed no decrease in total DNA content, implying no loss of cell number in these organs. However, DNA concentration in both tissues rose significantly, protein concentration fell only slightly, and total RNA levels remained unaltered. Thus, in these two organs, there was a selective loss of some cell constituents which reduced cell weight and accounted for the increased DNA concentration. These data, therefore, attest to the heterogeneous response of various organs, in terms of composition, to dietary thiamine deficiency.

As in the case of DNA synthesis, we cannot determine with certainty whether the compositional changes described above were due to thiamine deficiency directly, its nutritional effects, or both. Reversal of thiamine deficiency abolished most of the compositional changes in TD organs (liver DNA content remained depressed) but such reversal is also accompanied by increased food consumption. Other studies [20, 21] have shown that starvation alone depresses RNA and protein content of hepatocytes and that, in human liver, protein malnutrition increases DNA concentration [22], as was seen in our studies. Certainly, restriction of food, even in the absence of thiamine deprivation, resulted in compositional differences between the pair-fed and *ad lib.* fed controls which were in the same direction as in the thiamine-deficient rats (Tables 1–3). The administration of thiamine to symptomatic TD rats, while restricting food intake in these animals and their PFC controls, also did not resolve the problem fully. This procedure appeared to yield the same results in most instances as reversal accompanied by free food intake, suggesting that at least some of the compositional changes may be due to a direct thiamine action. For some data, however, fasting seemed to exert a different effect (i.e. heart protein concentration). This indicates that a firm resolution of this complex problem, which of course resembles the usual clinical pattern of thiamine deficiency, will require the use of simpler, perhaps *in vitro*, experimental systems.

REFERENCES

1. G. B. Phillips, M. Victor, R. D. Adams and G. S. Davidson, *J. clin. Invest.* **31,** 859 (1952).
2. M. Akbarian, N. A. Yankopaulos and W. H. Abelman, *Am. J. Med.* **41,** 197 (1966).
3. D. W. McCandless and S. Schenker, *J. clin. Invest.* **47,** 2268 (1968).
4. D. M. Dreyfus and G. Hauser, *Biochim. biophys. Acta* **104,** 78 (1965).
5. F. Novello and P. McLean, *Biochem. J.* **107,** 775 (1968).
6. S. J. Wolf, M. Brin and C. S. Davidson, *J. clin. Invest.* **37,** 1476 (1968).
7. G. I. Henderson, D. W. McCandless and S. Schenker, *Proc. Soc. exp. Biol. Med.* **144,** 596 (1973).
8. C. J. Gubler, *J. biol. Chem.* **236,** 3112 (1961).
9. G. I. Henderson and S. Schenker, *J. Lab. clin. Med.* **86,** 77 (1975).
10. M. J. Veen, G. Russell and G. H. Beaton, *Can. J. Biochem.* **41,** 2463 (1963).
11. T. R. Castles, S. Cambell, R. Gauge and C. C. Lee, *J. Pharmac. exp. Ther.* **181,** 399 (1972).
12. K. Burton, *Biochem. J.* **62,** 315 (1956).
13. Z. Dische, in *The Nucleic Acids*, p. 285. Academic Press, New York (1955).
14. E. F. Hartree, *Analyt. Biochem.* **48,** 422 (1972).
15. C. C. Liang, *Biochem. J.* **146,** 739 (1975).
16. P. R. Dallman and E. C. Manies, *J. Nutr.* **103,** 1311 (1973).
17. J. K. Chesters, in *Proceedings of the Nutritional Society*, p. 1. Two Hundred and Twenty-fifth Scientific Meeting, Eighty-Ninth Scottish Meeting, Sterling University (1970).
18. G. A. Sterling, J. Laughlin and L. A. Washington, *Expl. molec. Path.* **19,** 44 (1973).
19. S. Schenker, D. Chen, V. Speeg, C. Walker and D. McCandless, *Am. J. Digestive Dis.* **16,** 255 (1971).
20. M. F. Harrison, *Biochemistry* **55,** 204 (1953).
21. H. N. Munro, *Fedn Proc.* **27,** 1231 (1968).
22. R. Umaña, *Can. J. Biochem.* **44,** 455 (1966).