BIOCHEMICAL CHANGES IN LIVER ASSOCIATED WITH KWASHIORKOR [1]

By HELEN B. BURCH,[2] GUILLERMO ARROYAVE,[3] RUTH SCHWARTZ,[4] ANA MARIA PADILLA,[3] MOISÉS BĚHAR,[3] FERNANDO VITERI,[3] AND NEVIN S. SCRIMSHAW [3]

(From the Department of Pharmacology, Washington University School of Medicine, St. Louis, Missouri, and the Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemela)

(Submitted for publication June 10, 1957; accepted July 18, 1957)

Kwashiorkor, the most prevalent form of severe protein malnutrition, is a serious disease, often fatal to young children and especially common from weaning to five years of age. There are insufficient data concerning the chemical changes which take place during development of the disease, although clinical information is extensive. Present knowledge about various aspects of the disease has been reviewed by Trowell, Davies, and Dean (1), by Brock (2, 3) and more recently by Scrimshaw and co-workers (4). Its biochemical characteristics include low levels of serum protein, amylase, alkaline phosphatase, pseudo-cholinesterase, cholesterol, riboflavin and vitamin A. Concomitantly the livers are usually found to be high in fat and low in protein.

Waterlow (5, 6) did pioneering work on enzymes in liver biopsy specimens from malnourished children and reported that hepatic lactic dehydrogenase and cytochrome oxidase of four children in Gambia remained virtually unchanged after treatment whereas pseudo-cholinesterase was initially low but more than doubled on treatment. Later, Waterlow and Patrick (7, 8) studied levels of eight enzymes in liver biopsy samples from a large number of malnourished children in Jamaica on admission to the hospital and after treatment. For cytochrome oxidase, lactic, malic and glutamic dehydrogenases, succinoxidase, DPNH-cytochrome C reductase and transaminase they report unchanged activity following treatment. The only enzyme found to be reduced in activity in the disease was non-specific cholinesterase, which increased on treatment.

Extensive dietary studies in various parts of the world where children suffer from kwashiorkor reveal inadequate intake of protein as well as low dietary levels of other essential nutrients (1, 3). The protein is often of poor quality and therefore unfavorable for the synthesis of tissue protein. If dietary situations exist such that protein and other nutrients necessary for the synthesis of hepatic enzymes are inadequate, the levels of some enzymes in the liver should reflect the lack.

Certain hepatic flavin enzymes, particularly xanthine oxidase (9, 10), D-amino acid oxidase (11, 12), and glycolic acid oxidase (12), are greatly decreased either by riboflavin, protein or caloric deficiency in rats. Xanthine oxidase is also lowered by the lack of a single essential amino acid in the diet (13, 14). The levels of these flavoproteins and of riboflavin might conceivably be related to some of the changes in metabolism which occur in the livers of children with diseases of malnutrition, particularly where dietary protein and riboflavin are low.

This report gives results of biochemical measurements on liver biopsy samples from Guatemalan children with kwashiorkor. Analyses were made for xanthine, D-amino acid, and glycolic acid oxidases, DPNH-dehydrogenase, malic dehydrogenase, transaminase, riboflavin, total oxidized pyridine nucleotides, cholesterol, lipid and protein in the liver as well as the protein, cholinesterase and amylase in serum and riboflavin in red blood

[1] This investigation was supported in part by the National Science Foundation, The Williams-Waterman Fund for the Combat of Dietary Diseases, the Nutrition Foundation, Inc., and National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health (A-981), Public Health Service. Reported in part at the annual meeting of the American Society of Biological Chemists, April, 1957. INCAP Scientific Publication I-86.

[2] Pan American Sanitary Bureau Consultant to INCAP from July 28 to September 9, 1956.

[3] Staff members of the Institute of Nutrition of Central America and Panama (INCAP).

[4] Fellow of the World Health Organization of the United Nations.

cells. Samples were obtained from 13 children, in six cases both before and after treatment. For comparison, levels in liver specimens at autopsy from North American children dying of causes unrelated to kwashiorkor were also measured. To assess possible postmortem effects the livers of normal rats were assayed at various intervals after death.

EXPERIMENTAL METHODS

Sampling procedures. The children were selected as cases of typical kwashiorkor on admission to the General Hospital or the Hospital of the "Sociedad Protectora del Niño" of Guatemala City. If the prothrombin time of a blood sample proved satisfactory the initial biopsy sample was taken before any feeding or treatment was given. Franseen needle biopsies were done by aspiration through the pleural space after local anesthesia with procaine. The sample was put at once into an ice-cold tube which remained in ice until brought to the laboratory 30 to 45 minutes later.

The biopsy sample was rapidly weighed and dropped into a small glass homogenizer, containing 15 to 20 volumes of 0.02 M nicotinamide at 4° C., and thoroughly homogenized. Nicotinamide was used to prevent splitting of DPN by tissue DPNase. If the sample was large enough, about 5 mg. were taken for histological study. Each sample weighed 20 mg. on the average and provided enough tissue for the 12 analyses in duplicate or triplicate. Initial biopsy samples were obtained from 10 children. From six of these, biopsy samples were also obtained after three to four weeks of treatment. At this time the children were gaining weight and typical clinical signs of the disease such as edema, diarrhea, and skin lesions had disappeared. Although accurate dietary control was not possible to achieve in the wards of the General Hospital, the children usually received a mixed diet which included milk and supplementary vitamins. Adequate supervision of the diet was possible at the Hospital of the "Sociedad Protectora del Niño" where two children, M.A. and S.R., stayed. They received a diet rich in protein, particularly milk protein, eating gradually increasing amounts up to 5 grams of protein and 150 calories per kilo per day.

No liver samples from well-nourished Guatemalan children with no history of kwashiorkor were available for determinations of normal liver levels of chemical substances. However, to add to the group of recovered cases, biopsy samples were analyzed from three children clinically recovered from kwashiorkor, S.R., T.A. and I.T., from whom no initial samples were obtained. Another approximation of normal hepatic levels was obtained from liver at autopsy of children dying of causes unrelated to kwashiorkor in the St. Louis Children's Hospital. To determine possible consequences of postmortem delay, livers from a series of rats were analyzed at intervals of 0, 1, 6 and 10 hours after death from a blow on the head. The dead rats were allowed to remain at room temperature during the specified time intervals.

Analytical procedures. Labile enzymes were measured as soon as possible after sampling. DPNH-dehydrogenase was determined within 30 minutes after samples arrived in the laboratory. A spectrophotometric method was used which involved measurement of the change in optical density of DPNH at 350 mμ with potassium ferricyanide as the electron acceptor (12). D-amino acid and glycolic acid oxidases were measured by the method of Burch, Lowry, Combs, and Padilla (15) scaled to the use of 0.25 mg. of tissue (30 μl. incubation volumes). These methods depend upon the spectrophotometric measurement of the 3-hydrazinoquinoline derivatives of the α-keto acids formed during the action of the enzymes on D-alanine or glycolic acid, respectively, during a 30-minute incubation period. Xanthine oxidase was measured by the rate of oxidation of 2-amino-4-hydroxypteridine to fluorescent isoxanthopterin in the presence of 5×10^{-6} M methylene blue (12). This method completely avoids the usual troubles from tissue blanks. As a rule, 10 μl. of 1:20 homogenate, or 0.5 mg. of liver were used for each analysis. Riboflavin coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) were determined on the first few biopsy samples and the autopsy samples by a published fluorometric procedure (16). On later biopsy samples only the total riboflavin was measured. Oxidized pyridine nucleotides (PN) were determined after trichloroacetic acid precipitation of protein by a fluorometric procedure of Lowry, Roberts, and Kapphahn (17). Protein was determined colorimetrically (18).

The more stable enzymes and other substances were measured on frozen aliquots of the homogenates. For malic dehydrogenase (MDH) and aspartic-glutamic transaminase, methods developed for brain enzymes (19, 20) were adapted to liver samples. MDH was allowed to reduce oxalacetate with DPNH, and the DPN+ formed was measured by its fluorescence in alkaline solution. Transaminase was allowed to act on α-keto glutarate and aspartate in the presence of DPNH and an excess of purified pig heart MDH. The oxalacetate produced immediately oxidized DPNH to DPN+ which was measured fluorometrically. For each determination of MDH 0.15 γ of human liver or 0.08 γ of rat liver were incubated in a 12-μl. volume, and for each determination of transaminase 5 γ of human or rat liver were incubated in 50 μl.

Cholesterol in 0.5-mg. liver samples was estimated by the fluorometric method of Albers and Lowry (21) modified by McDougal and Farmer (22) for serum. Good reproducibility and 98 to 100 per cent recovery of added cholesterol were obtained. Lipids were measured on samples of similar size. Extraction was accomplished with 3:1 alcohol-ether mixture by adding 10 volumes for each of three extractions. The solvents were evaporated in a water bath at 90° C. and finally the last traces were removed in a special vacuum desiccator (21). The residue was extracted with ether; the ether was evaporated

TABLE I

Hepatic riboflavin and flavin enzymes in kwashiorkor, initially (I) and after treatment (T) *

Case	Sex and age *yrs.*	Clinical severity	Time treated *days*	Total riboflavin *mg./Kg.p*		Xanthine oxidase *mM/Kg.p/hr.*		D-amino acid oxidase *mM/Kg.p/hr.*		Glycolic acid oxidase *mM/Kg.p/hr.*		DPNH-dehydrogenase *M/Kg.p/hr.*	
				I	T	I	T	I	T	I	T	I	T
P. G.†	F–2.0	sev.	4	26		0.0		55		71		4.2	
R. O.†	M–5.5	mod.	10	137		1.7		134		473		17.5	
M. J.	F–3.0	mod.	12	138		1.6		78				20.1	
L. A.	F–7.0	mod.	17	108		3.5		147		362		16.3	
Z. A.	F–3.8	mod.	26	123	109	1.5	7.9	114	581	485	527	20.2	15.5
M. A.	M–4.0	mod.	48	155	109	4.0	5.2	168	552	500	524	24.2	15.4
R. V.	F–1.8	mod.	28	142	99	3.1	7.6	218	444	764	456	12.7	15.5
M. C.	F–1.7	mod.	29	92	122	2.7	5.4	179	430	342	509	15.0	14.4
L. S.	F–4.0	sev.	29	113	106	3.9	6.4	216	218	506	221	19.6	12.6
L. C.	F–3.0	sev.	26	103	115	1.2	5.2	96	421	333	516	21.0	17.2
S. R.	M–1.5	mod.	60		138		10.5		433		639		17.6
T. A.	M–1.5	mod.	168		113		8.0		407		575		14.5
I. T.	M–3.3	sev.	71		103		5.6		402		587		15.6
	Mean			123	113	2.6	6.9	150	432	471	506	18.5	15.4
	S.E.			7	4	0.4	0.6	16	34	52	42	1.2	0.5

* Values expressed per Kg. of liver protein.
† These two children died in the hospital. Values for P. G. were much lower than all others and have been excluded from the averages.

off and the lipid determined by the colorimetric method of Bragdon (23) adapted for 8 to 100 μg. of lipid in a final volume of 0.4 ml. by Chiang, Gessert, and Lowry (24). Trimyristin (Distillation Products Industries) was used as the standard lipid. Quantitative recovery of added lipid and reproducibility were obtained in preliminary tests made with rat and human liver.

In serum, protein was measured by the gradient tube method of Lowry and Hunter (25), cholinesterase and amylase by methods of Reinhold, Tourigny, and Yonan (26) and Smith and Roe (27). Red blood cell riboflavin on packed red cells was measured by a previously published method (28).

RESULTS

A. Clinical findings

Four of the children had severe kwashiorkor and the remainder were cases of moderate severity. All showed typical hair changes, skin lesions and edema. Apathy was a general characteristic, and all had diarrhea. Child P.G. was diagnosed initially as having very severe and apparently irreversible kwashiorkor and died four days after entering the hospital.

B. Hepatic flavin enzymes

All enzyme and coenzyme values have been calculated on the basis of protein. The data on riboflavin and four flavin enzymes (Table I) obtained on six children from whom biopsy samples were taken initially and after treatment revealed no significant rise in riboflavin, glycolic acid oxidase or DPNH-dehydrogenase. Remarkable increases are apparent in xanthine oxidase and D-amino acid oxidases. These findings are of special interest since, in the rat, hepatic xanthine oxidase falls with low dietary levels either of protein, calories or riboflavin, whereas DPNH-deyhdrogenase does not change in caloric restriction or riboflavin deficiency (12) until animals are near death. D-amino acid and glycolic acid oxidases in rat liver also fall if dietary riboflavin is absent but are not so greatly affected by low protein or calories as xanthine oxidase. Therefore, D-amino acid oxidase may not respond in the same manner to dietary restrictions in children with kwashiorkor as it does in rats.

Unusually low levels were obtained on child P.G. who failed to respond to treatment. Possibly the very low levels of these enzymes and other substances were associated with irreversible changes in the liver. The liver sample of P.G. was more fatty in gross appearance than any other. The DPNH-dehydrogenase is particularly interesting as it is the only really low value found for this enzyme.

The values in Table I indicate a highly significant increase in hepatic xanthine and D-amino acid oxidase levels calculated on the basis of liver protein of eight children before and after treatment of kwashiorkor. The other enzymes given in Tables I and II did not increase significantly relative to liver protein.

C. Hepatic substances other than flavin enzymes

Liver protein (Table II) increased on an average of 38 per cent ($p < 0.01$) during treatment in the six individuals studied both before and after therapy. At the same time total lipid fell to less than one-third the initial value, whereas cholesterol in the few samples tested showed no significant change. Two non-flavin enzymes, transaminase, and malic dehydrogenase, chosen for their significance in relation to amino acid metabolism and the citric acid cycle, respectively, were measured as controls of the flavin enzymes. Neither enzyme was changed significantly in the liver after treatment.

Pyridine nucleotides (DPN and TPN) appear to increase in most cases upon treatment although the increase is not statistically significant. These nucleotides are destroyed with exceptional speed by tissue and red cell enzymes and may have been partially split in some samples before analysis was possible. A change in extent of oxidation of these coenzymes would also affect the results. In rat liver samples, a decrease in total oxidized pyridine nucleotides PN occurred when they were kept in an ice bath at 4° C. for one hour without nicotinamide and analyzed by the technique used on the biopsy samples (Table IV). Therefore, the levels reported here are possibly somewhat low but should be comparable before and after treatment since the specimens were similarly handled, and enzyme action was stopped by adding aliquots to trichloroacetic acid at once.

D. Protein and enzymes in serum

A significant increase ($p < 0.01$) in serum protein on treatment (Table III) was accompanied by a significant rise ($p < 0.01$) in serum cholinesterase relative to protein. The increase in serum amylase was not significant. If activities of these enzymes are calculated per unit volume the increase become three-fold for cholinesterase and two-and-one-half-fold for amylase. Thus they would appear to have decreased similarly in kwashiorkor and to be synthesized at approximately the same rate upon recovery from the disease. It is obvious from the data, however, that the two enzymes behave differently relative to serum protein.

TABLE II

Liver concentrations of certain substances in kwashiorkor, initially (I) and after treatment (T) *

Case	Protein Gm./Kg.ω		Lipid Gm./Kg.ω		Cholesterol Gm./Kg.ω		Malic. dehyd. M/Kg.ω/hr.		Transaminase M/Kg.ω/hr.		Oxidized PN mM/Kg.ω	
	I	T	I	T	I	T	I	T	I	T	I	T
P. G.†	31						19		29		0.7	
R. O.	108		995				89		85		3.8	
M. J.	167						102		91			
L. A.	141						107		64		2.8	
Z. A.	124	150		257		23	101	132	96	76	2.8	5.9
M. A.	106	199	805		24		121	90	91	50	4.5	3.4
R. V.	87	192	833	250	28	25	176	113	91	60	3.4	4.1
M. C.	133	169	737	190	30	15	108	81	64	60	3.0	3.6
L. S.	121	167	731	219	15	24	122	105	84	56	4.1	4.6
L. C.	147	186					115	106	69	83	3.0	4.3
S. R.		144		360		26		124		118		5.3
T. A.		172		194		16		102		66		4.1
I. T.		191		199		18		100		70		3.8
Mean	126	174	820	238	24	21	116	106	82	71	3.4	4.4
S.E.	8	6	47	19	2	2	8	6	4	7	0.3	0.3

* Protein per Kg. of wet tissue; others expressed per Kg. of liver protein.
† Values for child P. G. were much lower than all others and have been excluded from the averages.

TABLE III

Levels of four constituents of blood serum or cells in kwashiorkor and the effects of treatment

Case	Serum protein Gm./100 ml.		Serum cholinesterase units/Gm. protein*		Serum amylase Smith-Roe units/ Gm. protein		Red cell riboflavin µg./100 ml.	
	I	T	I	T	I	T	I	T
P. G.	3.45†		275†		1.7†		24.2†	
R. O.	3.77		250		13.3		10.5	
L. A.	4.60		335		3.0		14.7	
Z. A.	4.10	6.68	330	540	8.3	11.1	11.2	18.6
M. A.	4.36	6.91	235	890	85.0‡	7.5‡	10.9	19.2
R. V.	4.50	7.68	455	935	2.9	15.0	11.0	
M. C.	3.50	6.00	270	550	3.1	8.0	23.8	27.8
L. S.	3.90	6.42	400	610	20.0	12.4	24.0	39.0
L. C.	3.74	7.48	515	660	8.3	11.4	13.9	24.2
S. R.								23.2
T. A.		6.40		820		4.5		
I. T.		6.75		715		14.8		26.2
Mean	4.06	6.79	350§	715§	8.4§	11.0§	15.0	25.4
S.E.	*0.14*	*0.20*	*30*	*55*	*2.3*	*1.5*	*2.0*	*2.3*

* The unit activity of the original serum as defined by Reinhold, Tourigny, and Yonan (26) multiplied by 5,000 and divided by Gm. protein per 100 ml.

† Omitted from the average as in previous tables.

‡ Child M. A. had hypertrophied salivary glands. These values have not been included in the average.

§ The average serum cholinesterase expressed as Michel Units is 0.28 ± 0.03 initially and 0.98 ± 0.09 after treatment; the serum amylase expressed as Smith-Roe Units per 100 ml. is 33 ± 9 and 76 ± 11, respectively.

Comparison of the increases in serum protein and cholinesterase with that of liver protein (Table II) suggests that serum cholinesterase and serum protein regenerate simultaneously with liver protein during recovery. The reports of Waterlow and others (1) that cholinesterase and protein in both liver and plasma are low in kwashiorkor and increase greatly on treatment are in accord with these results. The data on serum amylase are too variable to indicate any significant relation to protein in liver.

Red blood cell riboflavin increased 70 per cent on treatment although the liver riboflavin failed to show such a change. Few data are available for red cell riboflavin levels in young children, but for four children of similar ages an average of 30 γ per 100 ml. was previously reported (29). In adults, Bessey, Horwitt, and Love (30) have found the red cell level to be a reliable criterion of riboflavin deficiency, although in rats, at least, the liver is a more sensitive index of mild deficiency (31). Since there was no other evidence of riboflavin deficiency, the changes may be secondary to other alterations in the red cells.

E. Liver specimens at autopsy

Since information similar to that obtained from livers of children with kwashiorkor was not available for those of normal young children, measurements were made on liver specimens from St. Louis autopsies. Samples from five children who seemed to be well-nourished and who died after periods of one to four days in the hospital were analyzed. The time before samples were available varied from two to six hours after death. Levels obtained on the liver autopsy samples (Table IV) are in most instances similar to those obtained on liver biopsy samples of the cases of kwashiorkor after treatment. However, xanthine oxidase and glycolic acid oxidase activities averaged distinctly higher in the autopsy samples (100 and 50 per cent, respectively). The average xanthine oxidase value is five times greater than for the untreated children with kwashiorkor. Since glycolic acid oxidase in kwashiorkor did not change appreciably as the result of treatment, the low values among the children concerned may reflect some deficiency or disease state other than kwashiorkor. For example, this enzyme is a sensitive index of riboflavin deficiency (12).

TABLE IV

Enzyme activities of human and rat liver at autopsy *

Child† and age	Time to autopsy	Protein	FAD	FMN‡	Total riboflavin	Xanthine oxidase	D-amino acid oxidase	Glycolic acid oxidase	DPNH-dehyd.	Malic dehyd.	Trans-aminase	Oxidized PN
		Human liver										
	yr.	*hr.*	*Gm./Kg.w*	*mg./Kg.p*	*mg./Kg.p*	*mg./Kg.p*	*mM/Kg.p/hr.*	*mM/Kg.p/hr.*	*mM/Kg.p/hr.*	*M/Kg.p/hr.*	*M/Kg.p/hr.*	*M/Kg.p/hr.*	*mM/Kg.p/hr.*
R. J. B.,	3.0	6	155	99	7.4	106	8	271	562	10.8	121	53	1.8
E. H. L.,	2.0	3	120	91	11.1	102	9	396	892	15.2	115	53	4.8
W. G. H.,	7.0	2	175	90	13.7	104	14	351	732	17.8		65	3.1
R. J.,	2.5	3	162	68	4.3	72	18	505	617		281	110	3.7
D. A. Y.,	9.0	4	150			115	17	339	867	19.0	115	61	4.1
	Mean		152	87	9.1	100	13	372	734	15.7	158	68	3.5
	S.E.		*11*	*8*	*1.8*	*7*	*2*	*37*	*65*	*2*	*36*	*10*	*0.5*
		Rat liver										
No. of rats												
3		0	191§	150	14.0	164	48	814	920	35.0	200‖	86	4.9
			4	*5*	*1.4*	*6*	*2*	*27*	*94*	*2.3*		*2*	*0.5*
3		1¶	186	148	12.2	161	50	785	918	38.2			3.6
			4	*3*	*1.7*	*4*	*2*	*33*	*75*	*0.4*			*0.3*
3		1	187	132	19.0	151	43	874	720	38.9		76	4.1
			8	*8*	*2.0*	*10*	*2*	*76*	*135*	*1.2*		*5*	*0.4*
3		6	181	132	22.0	153	46	694	758	32.6			3.3
			5	*3*	*0.3*	*3*	*4*	*84*	*62*	*0.1*			*0.3*
3		10	181	128	26.0	154	43	730	877	29.7			2.7
			1	*3*	*2.3*	*7*	*2*	*42*	*74*	*0.6*			*0.4*

* Values are expressed as in Tables I and II. FMN and FAD are calculated as mg. riboflavin per kilo of protein.

† The causes of death were: acute bacterial meningitis, R. J. B.; pneumonia and cerebral edema of undetermined etiology with hypoxic nephrosis and moderate fatty metamorphosis of the liver, E. H. L.; tumor of the left lateral ventricle, W. G. H.; congenital heart disease with renal tubular acidosis and chronic congestion of the liver, R. J.; cerebral tumor, D. A. Y. Except for R. J., these cases had an acute onset of symptoms and died within a few days.

‡ The small amount of free riboflavin in liver is included in these values.

§ Figures are means with the standard error given below.

‖ This is the average of three adult rats.

¶ These analyses were done on liver samples from the first rats (zero time) after storage in a tube at 4° C. for one hour.

The rat liver analyses (Table IV) are presented as a confirmation of the validity of analyses on autopsy material. None of the four enzymes tested were diminished significantly within six hours of the death of the rat, and only one, DPNH-dehydrogenase, was changed within 10 hours. The coenzymes are not so stable; 10 to 15 per cent FAD was converted to FMN in 6 to 10 hours, and pyridine nucleotides were either hydrolyzed or reduced by 40 per cent in 10 hours. Human liver may not split FAD as rapidly as rat liver, however, since FMN was only 10 per cent of FAD in spite of the time lag before obtaining specimens. It will be noted that total riboflavin and all four flavin enzymes are higher in rat liver than in human liver, although transaminase is only slightly higher in the rat.

DISCUSSION

Values for liver and serum enzyme activities have been expressed throughout on the basis of protein because of the well known changes in liver and serum protein concentrations during treatment of kwashiorkor.

Hepatic flavin enzymes

The flavin enzymes, xanthine and D-amino acid oxidase, which were found to be low in kwashiorkor have also been shown to be lowered in rat liver by either protein or riboflavin deficiency. Although glycolic acid oxidase is greatly diminished in rat liver by riboflavin deficiency, it remains unchanged in human liver with treatment of kwashiorkor.

DPNH-dehydrogenase, however, is not altered in either the liver of children with kwashiorkor or in the tissues of rats during riboflavin deficiency or caloric restriction. Wainio and co-workers (32) report that DPNH-cytochrome C reductase falls in protein deficiency in the rat, but this has not been confirmed in this laboratory (12). Its failure to fall in these livers, except in the child P.G. who died, supports the idea that this is one of the most crucial of known flavin enzymes and one of those most firmly held by liver tissue even in various deficiency states.

This seeming paradox of finding no fall in riboflavin, relative to protein in liver from kwashiorkor cases when some flavin enzymes have dropped, is resolved by the fact that these enzymes account for an exceedingly small fraction of the total flavin present in liver.

Other substances in the liver

It is of interest that cholesterol does not increase in the liver in kwashiorkor according to the values presented here. This is contrary to the few data available on cases of kwashiorkor from India (33) in which much higher initial levels were found and a decrease with treatment was noted.

The finding of virtually unchanged levels of hepatic malic dehydrogenase and transaminase activity upon treatment of kwashiorkor agrees with Waterlow and Patrick (7). However, the absolute activity found by these investigators for transaminase is only one-fifteenth of the level reported here. This discrepancy may be traced to differences in methods used. These workers also found rat liver to be seven times lower in transaminase activity than human liver. In the method used here, oxalacetate produced is immediately reduced to malate, which prevents approach to equilibrium and consequent slowing of the reaction. Without means for removing one of the products, larger samples as may have been used by Waterlow and Patrick (7) in their studies of rat liver would give lower relative values.

It is now possible to measure a large number of enzymes with relative ease in the amount of material obtained by liver needle biopsy specimens. Through study of alterations in the enzymes, a better understanding of the metabolic changes in diseased tissues may be achieved. Changes in these functional proteins are particularly relevant to a deficiency disease such as kwashiorkor.

SUMMARY

1. Chemical changes in liver and serum during treatment of 13 children with kwashiorkor are reported. Results of similar analyses on liver autopsy specimens from five St. Louis children dying from causes unrelated to kwashiorkor are also included, together with analyses of livers from 12 rats, to confirm the validity of studying autopsy material.

2. The increase in protein and the decrease in total lipid with treatment were marked. Concomit-

antly there were striking increases relative to protein in xanthine oxidase and D-amino acid oxidase.

3. During treatment of the children with kwashiorkor no significant changes *relative to protein* were found in the following substances in liver: riboflavin, glycolic acid oxidase, DPNH-dehydrogenase, malic dehydrogenase, transaminase, oxidized pyridine nucleotides, and cholesterol.

4. The levels of all substances measured in the livers of kwashiorkor cases after treatment were generally equal to those of autopsy specimens of St. Louis children, except for glycolic acid oxidase and xanthine oxidase which did not increase to the values found in the autopsy samples.

5. Protein in serum increased 70 per cent during treatment and relative to protein, cholinesterase increased 100 per cent and amylase 30 per cent. The red blood cell riboflavin doubled.

ACKNOWLEDGMENTS

The autopsy specimens were made available through the diligent efforts of Dr. Donald B. Strominger, to whom the authors are greatly indebted. The authors wish to express their appreciation to Dr. Oliver H. Lowry for his exceedingly helpful advice and encouragement during this study. They also gratefully acknowledge the assistance of Miss Laura Herradora and Mrs. Loty de Funes in the analyses on serum.

REFERENCES

1. Trowell, H. C., Davies, J. N. P., and Dean, R. F. A., Kwashiorkor. London, Edward Arnold Ltd., 1954.
2. Brock, J. F., Survey of the world situation on kwashiorkor *in* Conference on Nutritional Factors and Liver Diseases, Annals of the New York Academy of Sciences. New York, New York Academy of Sciences, 1954, vol. 57, p. 696.
3. Brock, J. F., Nutrition *in* Annual Review of Biochemistry. Stanford, Annual Reviews, 1955, vol. 24, p. 523.
4. Scrimshaw, N. S., Béhar, M., Arroyave, G., Viteri, F., and Tejada, C., Characteristics of kwashiorkor (Sindrome pluricarencial de la infancia). Federation Proc., 1956, 15, 977.
5. Waterlow, J. C., Observations on the activity of some enzymes in the human liver. West Indian M. J., 1951, 1, 41.
6. Waterlow, J. C., Enzyme activity in human liver *in* Liver Injury, Transactions of the Eleventh Conference, F. W. Hoffbauer, Ed. New York, Josiah Macy Jr. Foundation, 1953, p. 72.
7. Waterlow, J. C., and Patrick, S. J., Enzyme activity in fatty livers in human infants *in* Conference on Nutritional Factors and Liver Diseases, Annals of the New York Academy of Sciences. New York, New York Academy of Sciences, 1954, vol. 57, p. 750.
8. Protein Malnutrition; Proceedings of a conference in Jamaica (1953) sponsored jointly by the Food and Agriculture Organization of the United Nations, the World Health Organization and the Josiah Macy Jr. Foundation, J. C. Waterlow, Ed. Cambridge, Cambridge Univ. Press, 1955, p. 16.
9. Axelrod, A. E., and Elvehjem, C. A., The xanthine oxidase content of rat liver in riboflavin deficiency. J. Biol. Chem., 1941, 140, 725.
10. Richert, D. A., and Westerfeld, W. W., Some interrelations of dietary protein, molybdenum, riboflavin and calories on liver and intestinal xanthine oxidase. Proc. Soc. Exper. Biol. & Med., 1953, 83, 726.
11. Axelrod, A. E., Sober, H. A., and Elvehjem, C. A., The D-amino acid oxidase content of rat tissues in riboflavin deficiency. J. Biol. Chem., 1940, 134, 749.
12. Burch, H. B., Lowry, O. H., Padilla, A. M., and Combs, A. M., Effects of riboflavin deficiency and realimentation on flavin enzymes of tissues. J. Biol. Chem., 1956, 223, 29.
13. Williams, J. N., Jr., and Elvehjem, C. A., The relation of amino acid availability in dietary protein to liver enzyme activity. J. Biol. Chem., 1949, 181, 559.
14. Williams, J. N., Jr., and Elvehjem, C. A., The effects of tryptophan deficiency upon enzyme activity in the rat. J. Biol. Chem., 1950, 183, 539.
15. Burch, H. B., Lowry, O. H., Combs, A. M., and Padilla, A. M., A sensitive method for measuring amino acid oxidases. Apr., 1956, Abstracts, Div. of Biol. Chem., Am. Chem. Soc., 129th meeting, Dallas.
16. Burch, H. B., Fluorimetric assay of FAD, FMN, and riboflavin *in* Methods in Enzymology, S. P. Colowick and N. O. Kaplan, Eds. New York, Academic Press Inc., 1957, vol. III, p. 960.
17. Lowry, O. H., Roberts, N. R., and Kapphahn, J. I., The fluorometric measurement of pyridine nucleotides. J. Biol. Chem., 1957, 224, 1047.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., Protein measurement with the Folin phenol reagent. J. Biol. Chem., 1951, 193, 265.
19. Lowry, O. H., Roberts, N. R., and Lewis, C., The quantitative histochemistry of the retina. J. Biol. Chem., 1956, 220, 879.
20. Lowry, O. H., Roberts, N. R., and Chang, M. L. W., The analysis of single cells. J. Biol. Chem., 1956, 222, 97.
21. Albers, R. W., and Lowry, O. H., Fluorometric determination of 0.1 to 10 micrograms of cholesterol. Anal. Chem., 1955, 27, 1829.
22. McDougal, D. B., Jr., and Farmer, H. S., A fluorometric method for total serum cholesterol. J. Lab. & Clin. Med., 1957, 50, 485.

23. Bragdon, J. H., Colorimetric determination of blood lipids. J. Biol. Chem., 1951, 190, 513.
24. Chiang, S. P., Gessert, C. F., and Lowry, O. H., Personal Communication.
25. Lowry, O. H., and Hunter, T. H., The determination of serum protein concentration with a gradient tube. J. Biol. Chem., 1945, 159, 465.
26. Reinhold, J. G., Tourigny, L. G., and Yoman, V. L., Measurement of serum cholinesterase activity by a photometric indicator method. Together with a study of the influence of sex and race. Am. J. Clin. Path., 1953, 23, 645.
27. Smith, B. W., and Roe, J. H., A photometric method for the determination of α-amylase in blood and urine, with use of the starch-iodine color. J. Biol. Chem., 1949, 179, 53.
28. Burch, H. B., Bessey, O. A., and Lowry, O. H., Fluorometric measurements of riboflavin and its natural derivatives in small quantities of blood serum and cells. J. Biol. Chem., 1948, 175, 457.
29. Snyderman, S. E., Ketron, K. C., Burch, H. B., Lowry, O. H., Bessey, O. A., Guy, L. P., and Holt, L. E., Jr., The minimum riboflavin requirement of the infant. Nutrition, 1949, 39, 219.
30. Bessey, O. A., Horwitt, M. K., and Love, R. H., Dietary deprivation of riboflavin and blood riboflavin levels in man. J. Nutrition, 1956, 58, 367.
31. Lowry, O. H., Biochemical evidence of nutritional status. Physiol. Rev., 1952, 32, 431.
32. Wainio, W. W., Eichel, B., Eichel, H. J., Person, P., Estes, F. L., and Allison, J. B., Oxidative enzymes of the liver in protein depletion. J. Nutrition, 1953, 49, 465.
33. Ramalingaswami, V., Sriramachari, S., and Tulpule, P. G., Hepatic cholesterol content in nutritional disorders. Lancet, 1952, 2, 661.