There was little evidence under the conditions of our experiment that the presence of factors in liver concentrate other than the vitamins mentioned above exert any appreciable effect upon the repletion of the depleted rat's tissue and blood proteins.

These results strengthen our view that antibody production depends upon an adequate supply of essential amino acids coming either from food or the animal's own protein stores. When both of these sources are markedly diminished the antibody producing capacity is markedly reduced. This capacity can be re-established by feeding adequate and well balanced quantities of amino acids coming from food protein, enzymatic protein hydrolysates, or amino acid mixtures.

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Increased Serum Phosphatase and "Hyperprothrombinemia" in Infectious Hepatitis of Children.

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The alkaline phosphatase activity of serum and the plasma prothrombin time are among the most commonly used indices of hepatic function. The phosphatase finds its most common application in the differential diagnosis of jaundice, where some observers believe the level of enzyme activity to be one of the most useful criteria in the differentiation of obstructive from hepatogenous jaundice.[1-3] Others,[4-8] while confirming that marked increases of phosphatase activity are more frequently found in obstructive than in "catarrhal" disorders of the liver, have observed overlapping to a considerable extent between the 2 groups. As to the behaviour of serum phosphatase in infectious hepatitis, results and conclusions of different observers are somewhat at variance. That some elevation of phosphatase occurs, appears evident from the figures of most studies.[1-9] While according to some, the increase of enzyme activity is only slight,[1-3] others[4-7,9] have frequently found greater rises. It has been suggested[1,2,5] that children may react during infectious hepatitis with higher phosphatase levels than adults, a suggestion in keeping with the results of one study, the subjects of which were for the most part children.[9]

In the study of the plasma prothrombin time, the greatest attention has been paid to the occurrence of hypoprothrombinemia. Lowering of the plasma prothrombin in patients with hepatic disease may be caused by a deficiency of vitamin K owing to decreased intestinal absorption or by impairment of the ability of the liver to form prothrombin. Two practical applications of the prothrombin time have been in the foreground: 1. as an index of bleeding tendency, and 2. as a

1 Roberts, W. M., *Brit. M. J.*, 1933, **1**, 734.

2a Flood, C. A., Gutman, E. B., and Gutman, A. B., *Arch. Int. Med.*, 1937, **59**, 981; 2b Gutman, A. B., Olson, K. B., Gutman, E. B., and Flood, C. A., *J. Clin. Invest.*, 1940, **19**, 129; 2c Gutman, A. B., and Hanger, F. M., Jr., *M. Clin. North America*, 1941, **25**, 837.

3 Rothman, M. M., Meranze, D. R., and Meranze, T., *Am. J. Med. Sc.*, 1936, **192**, 526.

4 Herbert, F. K., *Brit. J. Exp. Path.*, 1935, **16**, 365.

5 Shay, H., and Fieman, P., *Am. J. Digest. Dis.*, 1938, **5**, 597.

6 Giordano, A. S., Wilhelm, A., and Prestrud, M. C., *Am. J. Clin. Path.*, 1939, **9**, 226.

7 Greene, C. H., Shattuck, H. F., and Kaplowitz, L., *J. Clin. Invest.*, 1934, **13**, 1079.

8 Cantarow, A., and Nelson, J., *Arch. Int. Med.*, 1937, **59**, 1045.

9 Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 107.

TABLE I.
Indices of Hepatic Function in Infectious Hepatitis of Children.

Case No.	Age, yrs	Symptoms before onset of jaundice	Serum: Approximate time since onset of jaundice, days	Serum: Bilirubin, mg per 100 cc	Serum: Cephalin flocculation	Serum: Thymol turbidity, units	Serum: Phosphatase, Bodansky units per 100 cc	Plasma: Prothrombin time undil. seconds	Plasma: Prothrombin time dil. const. seconds
1	9	6 days prior, vomiting	1	3.8	+++	8		16.5	4.1
			2		+++	12		21.8	3.6
			6	1.2	+++	12	15	33.6	6.3
			12	0.7	++	5	5	19.6	3.7
2	11	5 days prior, abdominal pain	1	3.8	+++	13		20.9	3.6
			4	2.1	+++	16	16	23.5	5.4
			8	1.0	++++	20	8	22.2	4.4
			16	0.2	+++	10	5		
3	10	8 days prior, epigastric pain and vomiting	3	6.9	+++	22	14	18.8	2.4
			7	2.0	+++	20	12	25.2	3.1
			15	1.0	++++	12	5	27.0	3.9
4	14	10 days prior, anorexia and fever	1	4.8	+++	20	17	17.8	3.6
			3	5.2				18.6	3.6
			6	3.8	+++	20	15	25.2	5.2
			26	0.3	++		6	20.2	3.6
5	13	2 days before examination, fever. Bilirubinuria on admission	0	1.0	+++	20			
			3	0.7	+++	15	19		
			5	0.7	++++		12	23.3	4.8
			8	0.6			10	27.3	5.2
6	10	7 days prior, fever	**1**	8.5	+++			14.7	2.7
			4	4.0			37	16.8	2.4
			7	2.3	+++			18.7	3.3
			10	1.8			8	25.3	3.8
7	9	6 days prior, fever, nausea	1	4.9	+++		26	16.4	1.3
			21	0.5	++		8	19.8	4.0
8	9	6 days prior, headache and abdominal pain	1	2.7	+++		17	16.4	2.1
			26	0.5	++		6		
9	10	None	8	3.0	++++		15	17.1	
			23	0.5	++		7		

10	11	4 days prior, epigastric discomfort	2	11.8	++	18	16.0	0.5	
			10	2.7		8			
11	7	5 days prior, epigastric pain and vomiting	1	4.8	++		16.8	2.9	
			2				17.0	2.5	
12	14	8 days prior, fatigue and nausea	5	7.8	+++	25	20.6	0.8	
			15	0.7	+++	7			
13	11	8 days prior, epigastric pain; 3 days prior, epistaxis	1	4.4	++		16.1	2.4	

measure of hepatic function, when vitamin K is supplied in adequate amounts. In infectious hepatitis, moderate degrees of hypoprothrombinemia have been found. A common observation is that, when the disease is severe, the usually present hypoprothrombinemia does not respond readily to vitamin therapy.

In this communication are presented observations on 13 children on whom determinations of phosphatase and prothrombin time, together with other tests of liver function, were obtained in the early stages of infectious hepatitis. In these cases transient elevations of considerable magnitude in the activity of phosphatase were observed. In the majority of instances abnormally short prothrombin times were noted during the first days of illness. These soon gave way to prolonged prothrombin times. The findings, aside from their limited practical value, are of interest in the consideration of the mechanism and the pattern of change of hepatic function in disorders of the liver.

Material and Methods. The patients studied were 13 children, 7 of whom were girls, varying in age from 7 to 14 years. Before the onset of hepatitis they had been well, and they gave no history of relevant antecedent disease. Their symptoms were mild in nature, and complaints were elicited in some only on questioning at routine visits to the outpatient department. Generally, 2 to 8 days before the onset of jaundice untoward manifestations, such as fever, anorexia, abdominal pain, or vomiting had occurred. Eight of the children were examined the day after the jaundice had been noticed, others 2, 3, 5 and 8 days afterwards. One patient, who may be classified as having exhibited hepatitis without jaundice, never presented clinical icterus. Bilirubinuria was found in all instances. The stools of 2 of the patients appeared lighter in color than normal.

Serum bilirubin was determined according to the method of Malloy and Evelyn,[10] alka-

[10] Malloy, H. T., and Evelyn, K. A., *J. Biol. Chem.*, 1937, **119**, 481.

[11] Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.

[12] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

line serum phosphatase according to the procedure of Bodansky,[11] except that the method of Fiske and Subbarow,[12] modified for the photoelectric colorimeter, was used to measure the inorganic phosphate. Prothrombin time was determined by a slightly modified method of Fullerton,[13] with Russel viper venom as thromboplastin. The prothrombin time of undiluted plasma as well as of several plasma dilutions was tested. The data of the dilution curves for each plasma were expressed by 2 numbers: 1. the clotting time of undiluted plasma, and 2. the dilution constant expressing the increase in seconds per unit increment of reciprocal dilution. The use of a constant is based on the finding of a linear relationship between the clotting time and the reciprocal of the dilution for any given plasma (unpublished data). The mean normal value, based on the data of 40 subjects, for the prothrombin time of undiluted plasma was 19.8 sec. $\pm$ a standard deviation of 1.2 sec. and for the dilution constant 3.8 $\pm$ 0.8 sec. Accepting the customary limits of $\pm$ 2 $\times$ S.D. as the normal range, values below 17.5 and over 22.3 seconds for the undiluted plasma clotting time and values below 2.1 and over 5.5 for the dilution constant may be considered abnormal. The cephalin flocculation test was carried out and read according to Hanger,[14] with precautions to avoid exposure to light. Only the 24-hour readings were recorded. The thymol turbidity test was performed according to Maclagan.[15] Values over 4 units according to the author of the method are considered to be abnormal.

Results and Discussion. The high levels of phosphatase activity observed in the patients here presented are comparable to those reported on a group of patients of similar age distribution.[9] That some increase of enzyme activity, although perhaps of smaller degree, occurs in adult patients, is evident from the data of other studies.[1-8] The transient nature of the increase is apparent from the data on cases 1 to 3, and 5, in which normal values were found when the determinations were repeated 2 to 6 days after a high phosphatase value had been determined. The evanescent nature of the rise of phosphatase activity, although not stressed, is also indicated from an inspection of the data of previous reports.[2,9] The practical importance of these observations is limited, but they indicate that any interpretation of single determinations of phosphatase activity should be made cautiously.

The degree and pattern of the increase in phosphatase aside from its diagnostic aspect, is of interest in the consideration of the mechanism of this phenomenon. According to the "retention" theory,[2] most of the serum phosphatase activity is osteogenous. This view is based on the finding of high enzyme activity in bone tissue, and on changes observed in the serum enzyme activity in experimental conditions and disorders affecting the skeletal metabolism. The primary function of the liver, according to this theory, is excretion of the serum phosphatase, an assumption based on the high enzyme activity of bile. The elevation of phosphatase in obstructive jaundice is ascribed to interference with the enzyme excretion. By the same token, an elevated phosphatase level is taken as a sign of impairment of the excretory function of the liver. Another explanation[9] for the behaviour of the serum phosphatase is that at least part of the normal activity is hepatogenous, and that the rise in the enzyme activity in hepatic disease is caused by stimulation of the liver cell. The variable extent of the increase is explicable on the assumption that the formation of the phosphatase is variably influenced by different types of noxious influence on the liver cell. The data here reported appear to favor the view that the elevation of the enzyme activity represents a characteristic response of the liver to a certain type of damage. Its occurrence in the early phase of the disease, as well as the dissociation between bilirubin level and extent of increase of the enzyme activity, suggest that the rise in phosphatase reflects a stimulation rather than a suppression of the activity of the liver cell. The incidence of considerably increased

[13] Fullerton, L., *Lancet*, 1940, **2**, 195.

[14] Hanger, F. M., *J. Clin. Invest.*, 1939, **18**, 261.

[15] Maclagan, N. F., *Brit. J. Exp. Path.*, 1944, **25**, 234.

phosphatase activity, more frequent in obstructive than in hepatogenous jaundice, may be explained at least in part by the assumption that biliary obstruction in most instances represents a peculiarly effective stimulus to the liver cell. The finding of normal or only moderately increased phosphatase levels in infants with congenital atresia of the bile ducts[2a,2b,6,8] (unpublished data), indicates that the element of retention, at least in certain types of obstructive jaundice, may play only a minor role. In such patients, on the basis of the "retention" theory one would expect high phosphatase levels, since in this age period the serum phosphatase activity normally is higher than in adult life.

In 8 of the 13 patients abnormally short prothrombin times in undiluted plasma were observed in the first determination. The dilution constant was similarly reduced in most instances to the lower limits of normal, or below. Subsequent determinations, performed within short periods of time in cases 1 to 6, showed variable prolongation of the prothrombin time beyond the upper limits of normal. The interpretation of the abnormally short prothrombin times is as yet uncertain. Some observers have found reduced prothrombin times in animals following large doses of vitamin K,[16] and after the administration of methylated purines,[17] and they have interpreted their findings as an indication of the occurrence of hyperprothrombinemia. This view has been challenged, as such effects have not been noted when the original method of Quick was used.[18] At present it is still undetermined whether the abnormally short prothrombin times reflect an actual increase in prothrombin, or changes in the rate of its activation. An increase in the amount of activators might be involved, or a reduction of the normally occurring clotting inhibitors. If the present view that vitamin K is specifically concerned in the formation of prothrombin is correct, the transient occurrence of short clotting times after large doses of the vitamin,[16] and after therapeutic doses in patients with hypoprothrombinemia (see [16] for references, also unpublished data), would suggest that these changes involve prothrombin *per se*. Whatever the true explanation, the finding of transient "hyperprothrombinemia" indicates that the liver cell reacts to the virus of infectious hepatitis at first with stimulation, followed by depression according to a pattern characteristic for many biologic phenomena. The period of stimulation may be exceedingly short: for instance the prothrombin time in case 1 changed within one day from a low value to one at the upper limits of normal, and increased further to a greatly prolonged value within a few more days. An even more rapid transition was observed in a case of erythroblastosis fetalis (unpublished), where the prothrombin time increased within 12 hours from 8 to 25 seconds.

Summary. Changes of the alkaline phosphatase activity in serum and of plasma prothrombin time were studied in 13 children with infectious hepatitis. Considerable increases of phosphatase and abnormally short prothrombin times were found transiently during the early part of the disease. The "hyperprothrombinemia" soon was followed by hypoprothrombinemia. The findings are interpreted as an indication of stimulation of the liver cell during the first stages of infectious hepatitis.

[16] Field, J. B., and Link, K. P., *J. Biol. Chem.*, 1944, **156**, 739.

[17] Field, J. B., Larsen, E. G., Spero, L., and Link, K. P., *J. Biol. Chem.*, 1944, **156**, 725.

[18] Quick, A. J., *J. Biol. Chem.*, 1945, **161**, 33.