4. Kabat, E. A., and Mayer, M. M., *Experimental Immunochemistry,* p81, Charles C. Thomas, Springfield, Ill., 1948.

5. McDuffie, F. C., and Kabat, E. A., *J. Immunol.,* 1956, v77, 193.

6. Wardlaw, A. C., and Pillemer, L., *J. Exp. Med.,* 1956, v103, 553.

7. Blattberg, B., unpublished data, 1957.

Received July 3, 1957. P.S.E.B.M., 1957, v96.

Effect of Partial Hepatectomy on Liver and Kidney Coenzyme A Content in the Rat.* (23400)

ALVIN S. WENNEKER† (Introduced by R. E. Shank)

The rat liver regenerates rapidly after surgical extirpation of as much as two-thirds of the liver mass. During the regenerative process protein content and organ weight are restored to the original levels within a period of from 14 to 21 days after partial hepatectomy(1,2). Protein synthesis proceeds most rapidly in the first 4 to 8 days and affords the opportunity for study of protein anabolic processes in liver. The investigation described below was undertaken in the effort to evaluate the role of coenzyme A in this process. Acetyl coenzyme A is important in systems of carbohydrate and fat metabolism through mechanisms of utilization of 2 carbon fragments(3,4.5,6,7). It is to be presumed that similar mechanisms are involved in systems of protein metabolism. In this investigation the concentrations of coenzyme A in liver and kidney of rats were studied during the period of increased protein synthesis induced by subtotal hepatectomy.

Procedure. Male Sprague-Dawley rats weighing 95 to 135 g were divided into 4 groups. A subtotal hepatectomy was performed on 44 rats in Group I by excision of median and left lateral liver lobes *in toto* leaving the posterior and caudate lobes intact (major hepatectomy). The amount of liver removed was approximately 66% of total wet liver weight, a value corroborated by performing the identical operation on 10 additional animals which were immediately sacrificed and the excised and remaining liver mass determined. Rats in this group were sacrificed at intervals up to 14 days following major hepatectomy. Group II consisted of 10 rats which were subjected to excision of the left lobule of the median lobe of the liver (minor hepatectomy). Minor hepatectomy thus performed removed approximately 8.5% of total liver, or about 1/8th as much liver tissue as was removed at major hepatectomy (Group I). Animals in Group II were sacrificed 24 hours post-operatively and served as a control in evaluation of surgical stress and trauma to the liver on hepatic coenzyme A concentrations. Group III included 10 rats which were subjected to major hepatectomy and sacrificed 24 hours later, in order to determine the effect of early liver regeneration on kidney coenzyme A concentrations. Group IV consisted of 10 normal rats which were sacrificed and kidney coenzyme A concentrations determined. This group thus served as a control for Group III.

All animals were fed a stock diet (Purina Laboratory Chow) *ad libitum.* The daily food consumed by each rat in Group I was measured for one week prior to surgery and throughout the post-operative period daily intake of calcium pantothenate was estimated from amount of food consumed. Hepatectomized rats in Groups I, II and III received 15,000 units of procaine penicillin, intramuscularly, immediately following surgery. Body weights were measured at time of hepatectomy and at sacrifice. At the conclusion of the experimental periods, animals were decapitated. The remaining hepatic tissue and

* Supported in part by a grant from the Nutrition Foundation.

† Fellow of National Foundation for Infantile Paralysis.

both kidneys were removed and weighed.

Coenzyme A and protein analyses were performed on liver tissue removed at operation and at sacrifice of animals in Groups I and II and on kidney tissue obtained at the time of sacrifice of rats in Groups III and IV. Tissues were immediately blotted, weighed, and homogenized in 9 volumes of distilled water in a Potter-Elvehjem apparatus at 0°C. An aliquot of each homogenate was placed in boiling water for 5 minutes, then cooled in crushed ice. The cooled homogenates were centrifuged to remove precipitated material and an aliquot of supernatant solution analyzed for coenzyme A. Liver tissue obtained at hepatectomy was prepared in the above manner and the supernatant solutions were stored at −10°C until respective animals were sacrificed. Thus, coenzyme A assays were performed simultaneously on tissue removed at surgery and at sacrifice. The remainder of each homogenate was frozen and set aside for protein determination by the copper-Folin method of Lowry(8).

Coenzyme A analyses were performed by the arsenolysis method(9) with minor modification. Dilithium acetyl phosphate was prepared by the reaction of isopropenylacetate with phosphoric acid and purified by 3 precipitations with absolute ethanol(10). Crude phosphotransacetylase was obtained from Dowex-1 treated *Cl. kluyverii* cells.‡ Standard coenzyme A (Sigma Chemical Co.) providing 300 Lipmann units per mg was diluted with distilled water to obtain 10 units per ml. Analyses were performed on 0.3 ml of each tissue supernate and compared with the standard solution containing 3 units of coenzyme A. All solutions were read in the Coleman spectrophotometer at a wave length of 540 mμ. Coenzyme A concentrations were expressed as units per g wet tissue and as units per 100 mg tissue protein. Tissue protein concentration was considered a more suitable standard of reference than desoxypentosenucleic acid (DNA) since both increased and decreased hepatic DNA concentrations have been noted during early phases of liver regeneration(11).

Preliminary studies on 5 animals revealed coenzyme A concentrations to vary less than 8% between the median and posterior liver lobes, a difference which was not statistically significant ($p>0.1$). Thus, the possibility that inherent differences in coenzyme A concentration between liver lobes could account for the changes found was excluded.

Results. The majority of animals subjected to surgical procedures demonstrated weight loss during the first 2 to 3 post-operative days (Table I). A portion of this loss was attributed to removal of liver tissue at surgery, but the major loss of body mass was due to decreased food intake. During the first 24 hours following surgery, calcium pantothenate intake varied from 0.033 to 0.111 mg, with a mean of 0.090 ± 0.007 mg. During the next 24 hours, mean calcium pantothenate intake was 0.164 ± 0.016 mg, with a minimum of 0.135 mg. The latter values far exceeded minimal daily requirements reported for rats(12,13).

Rates of liver regeneration following major hepatectomy followed fairly closely those previously reported(2,14). Kidney weights were unaffected by major hepatectomy. Liver coenzyme A concentrations in Groups I and II are summarized in Table I. During the first 3 days following major hepatectomy (Group I), coenzyme A concentrations were significantly diminished in regenerating liver tissue when compared to levels present in liver tissue removed at time of partial hepatectomy. Decreases were apparent when coenzyme A concentration was based upon either wet tissue weight or upon tissue protein concentration. The lowest coenzyme A concentrations were observed 24 hours after surgery. By the fifth post-operative day, coenzyme A concentrations in regenerating livers were only slightly less than in their surgically removed counterparts, the differences attaining a low degree of significance. On the 8th and 14th days, coenzyme A levels in surgically removed and regenerating liver tissue were not significantly different. The slight decrease in hepatic coenzyme A concentration noted 24 hours after minor hepatectomy (Group II)

‡ Extraction and Dowex-1 treatment of the dried cells were carried out by the Sigma Chemical Co., St. Louis, Mo.

TABLE I. Liver Coenzyme A Content* in Partially Hepatectomized Rats.

Days regeneration	No. rats	Mean body wt (g) Initial	Final	Units/g wet liver Initial‡	Final§	% change	Significance of change (p value)	Units/100 mg tissue protein Initial	Final	% change	Significance of change (p value)
					Group I (major hepatectomy)						
1	10	120	115	142.2 ± 11.3†	99.4 ± 5.7	-30.0	<.001	86.8 ± 6.8	69.2 ± 4.3	-20.2	<.005
2	7	125	116	147.0 ± 10.2	106.5 ± 8.8	-27.6	<.02	86.5 ± 5.2	71.3 ± 5.5	-17.6	<.05
3	6	117	108	153.8 ± 5.2	118.3 ± 7.7	-23.1	<.005	93.7 ± 1.9	77.3 ± 3.8	-17.5	<.005
5	8	102	113	127.2 ± 9.1	112.5 ± 9.1	-11.6	>.05	73.2 ± 3.5	66.1 ± 4.6	- 9.7	>.05
8	8	110	137	140.5 ± 12.6	142.2 ± 8.2	+ 1.2	>.90	84.3 ± 6.1	87.3 ± 4.5	+3.6	>.60
14	5	114	116	129.8 ± 14.7	140.6 ± 13.8	+ 8.3	>.10	78.8 ± 8.7	79.5 ± 8.5	+ .90	>.70
					Group II (minor hepatectomy)						
1	10	107	103	127.0 ± 6.5	113 ± 5.7	-11.0	<.02	75.8 ± 3.4	68.8 ± 2.9	- 9.2	<.005

* Expressed as Lipmann units. † S.E. of mean. ‡ At operation. § At necropsy.

was statistically significant. However, this decrease was only ⅓ (units per g wet tissue) to ½ (units per 100 mg of tissue protein) as great as that observed 24 hours after major hepatectomy. An analysis of variance in Groups I and II revealed the differences in response of hepatic coenzyme A levels to the 2 surgical procedures were highly significant ($p<<0.01$). The differences in coenzyme A concentrations of liver tissue removed at surgery in Group I series and Group II rats were not significant.

Kidney coenzyme A concentrations are presented in Table II. The mean coenzyme A concentration of kidney tissue 24 hours after major hepatectomy was slightly (16%) but significantly greater than that found in unoperated animals. Protein concentrations of the kidney and liver are presented in Tables II and III. Significant decreases in protein concentration were observed in regenerating livers of Group I during the first 3 post-operative days, as has been previously reported (15,16). Diminished hepatic protein concentration may result from an increase in hepatic fat and water content which has been observed early during liver regeneration(15, 16, 17) or from partial starvation(18,19). It should be noted, however, that no significant alterations in protein levels occurred in Group II livers nor in Group III kidneys.

Discussion. The results of these experiments clearly demonstrate a decrease in hepatic coenzyme A concentration during the first 3 to 5 days after partial hepatectomy. This period has been shown to be one in which liver regeneration proceeds most rapidly(2, 15). Factors which may result in decreased coenzyme A levels during this interval must be considered. It has been mentioned that an increase in fat and water contents of liver occur early in the regenerative period. The effects of fat infiltration and increased water content of hepatic tissue were minimized by expressing coenzyme A levels as units per 100 mg of tissue protein. Decreased coenzyme A concentrations remained significant when expressed in this manner. Therefore, it is unlikely that either fat or water content was of importance in lowering hepatic coenzyme A

TABLE II. Renal Coenzyme A and Protein Contents in Partially Hepatectomized and Non-Operated Rats.

	No. of rats	Body wt, g	Coenzyme A: Units/g wet tissue	Coenzyme A: Units/100 mg protein	Protein conc., mg/g wet tissue
Group III†	10	109	53.6 ± 1.4*	39.4 ± 1.1*	136 ± 2
" IV (control)	10	113	46.2 ± 2.3	33.9 ± 1.7	137 ± 2

* Difference from Group IV (control) significant, $p < .02$.
† Determinations 24 hr after two-thirds partial hepatectomy.

levels. A deficient intake of calcium pantothenate associated with partial starvation was noted in several animals on the first postoperative day only. Although an adequate intake of pantothenic acid is necessary to maintain normal tissue coenzyme A levels, it is improbable that starvation was a factor resulting in diminished hepatic coenzyme A concentrations in the present experiment. Ringler has shown that total starvation for 4 days had little effect upon liver coenzyme A levels in the rat(20).

Surgical stress and trauma to the liver may contribute to decreased hepatic coenzyme A concentrations since slight, but statistically significant, decreases in liver coenzyme A were observed after the stress of laparotomy and minor hepatectomy. However, these decreases were much less than those observed in the rapidly regenerating liver which remained after excision of two-thirds of the total liver. Therefore, stress and liver injury were not solely responsible for the marked reduction in liver coenzyme A concentration observed after major hepatectomy.

The lowered coenzyme A levels present in early liver regeneration may be associated with metabolic alterations which precede and accompany active tissue growth. Novikoff and Potter(11) demonstrated a marked lowering of certain hepatic Kreb cycle oxidases beginning as early as 15 hours and persisting 3 to 4 days after partial hepatectomy. This reduction in enzymes concerned with carbohydrate metabolism is paralleled closely both in degree and duration by the decreased coenzyme A concentrations present in rapidly regenerating liver tissue. In addition, markedly reduced coenzyme A levels have been demonstrated in actively growing induced rat liver tumors(21), paralleling an earlier observation that rat liver tumors oxidize oxaloacetic acid poorly(22). It becomes apparent that during rapid tissue growth and repair carbohydrate metabolism becomes subsidiary to protein synthesis, and that coenzyme A concentrations more closely parallel the former. If the tissue concentration of an enzyme is an index of its relative activity, the data suggest that coenzyme A may not be of major importance in the synthesis of tissue proteins.

TABLE III. Liver Protein Content in Partially Hepatectomized Rats.*

Days regeneration	Protein conc., mg/g wet liver: Initial	Protein conc., mg/g wet liver: Final	% change	Significance of change (p value)
		Group I		
1	164 ± 2	146 ± 2	−11	$<<.001$
2	170 ± 7	149 ± 4	−12	$<.02$
3	164 ± 3	153 ± 5	− 7	$<.05$
5	173 ± 6	170 ± 5	− 2	$>.90$
8	165 ± 4	163 ± 3	− 1	$>.90$
14	164 ± 4	179 ± 4	9	$>.10$
		Group II		
1	167 ± 3	164 ± 2	− 2	$>.30$

* Determinations on same animals charted in Table I.

No adequate explanation for the observed rise in renal coenzyme A concentration following major hepatectomy is suggested by the data presented. It is entirely possible that the rise is compensatory to loss of a substantial portion of the total body coenzyme A via hepatectomy.

Conclusion. 1. Following partial hepatectomy in the rat, liver coenzyme A concentrations were found to be significantly reduced throughout the period of rapid liver repair. Neither fat nor water infiltration of liver, partial starvation, nor surgical stress with injury to the liver were thought to be important contributing factors. Evidence suggested that the reductions were related to alterations in

carbohydrate metabolism which accompany rapid tissue growth and that coenzyme A may not play an important role in protein synthesis. 2. An unexplained rise in renal coenzyme A concentration was observed 24 hours after partial hepatectomy.

The author wishes to thank Dr. Robert E. Shank, under whom this project was carried out, and Dr. Albert B. Eisenstein for their helpful and generous advice and encouragement, and Dr. Arthur Kornberg for his kind donation of dried *Cl. kluyverii* cells used in the arsenolysis assay for coenzyme A.

1. Gurd, F. N., Vars, H. M., and Ravdin, I. S., *Am. J. Physiol.*, 1948, v152, 11.
2. Higgins, G. M., and Anderson, R. M., *Arch. Path.*, 1931, v12, 186.
3. Chow, T. C., and Lipmann, F., *J. Biol. Chem.*, 1952, v196, 89.
4. Klein, H. P., *Fed. Proc.*, 1951, v10, 209.
5. Klein, H. P., and Lipmann, F., *J. Biol. Chem.*, 1953, v203, 95; 1953, v203, 101.
6. Ochoa, S., Stern, J. R., and Schneider, M. C., *ibid.*, 1951, v193, 691.
7. Stern, J. R., and Ochoa, S., *ibid.*, 1951, v191, 161.
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *ibid.*, 1951, v193, 265.
9. Stadtman, E. R., Novelli, G. D., and Lipmann, F., *ibid.*, 1951, v191, 365.
10. Stadtman, E. R., and Lipmann, F., *ibid.*, 1950, v185, 549.
11. Novikoff, A. B., and Potter, V. R., *ibid.*, 1948, v173, 223.
12. Emerson, G. A., and Evans, H. M., PROC. SOC. EXP. BIOL. AND MED., 1941, v46, 655.
13. Unna, K., and Richards, G. V., *J. Nutrition*, 1942, v23, 545.
14. Wenneker, A. S., and Sussman, N., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 683.
15. Brues, A. M., Drury, D. R., and Brues, M. C., *Arch. Path.*, 1936, v22, 658.
16. Harkness, R. D., *J. Physiol.*, 1952, v117, 267.
17. Szego, C. M., and Roberts, S., *J. Biol. Chem.*, 1949, v178, 827.
18. Addis, T., Poo, L. J., and Lew, W., *ibid.*, 1936, v115, 117.
19. Kosterlitz, H. W., *J. Physiol.*, 1947, v106, 194.
20. Ringler, I., and Leonard, S. L., *Endo.*, 1954, v55, 363.
21. Higgins, H., Miller, J. A., Price, J. M., and Strong, F. M PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 462
22. Potter, V. R., Lepage, G. A., and Klug, H. L., *J. Biol. Chem.*, 1948, v175, 619.

Received July 3, 1957. P.S.E.B.M., 1957, v96.

Enzymic Action of Influenza Virus on Human Erythrocyte Stroma Components.* (23401)

CALDERON HOWE,† HARRY M. ROSE, AND LOUIS SCHNEIDER
Department of Microbiology, College of Physicians and Surgeons, Columbia University

Sialic acid, a compound originally isolated from bovine submaxillary gland by Blix(1) is now recognized as an important constituent of soluble inhibitors of influenza virus hemagglutination. Its structure, proposed by Gottschalk(2), has only recently been established by the work of Heimer and Meyer(3). This chromogenic substance has also been identified among the dialyzable split products resulting from the action of influenza virus on certain mucoids(4). Interest in sialic acid in relation to action of influenza virus has led to a reexamination of receptor substances in human erythrocytes. The present study is based on earlier work(5) in which it was shown that virus receptors and blood group antigens could be concentrated from lyophilized erythrocyte stroma by relatively simple ultracentrifugation methods into a lipid-rich carbohydrate-containing fraction. This fraction has now been found also to contain a measurable proportion of chromogenic material giving absorption spectra in the direct

* These studies were conducted under the auspices of the Commission on Influenza, Armed Forces Epidemiological Board, and were supported in part by the Office of the Surgeon General, Department of the Army, Washington, D.C., and the Philip Hanson Hiss Memorial Fund.

† Markle Scholar in Medical Science.