shown in Table II, after injecting radioactive phosphorus and preparing TCA filtrates.

Summary. 1. Most of the enzymes of glycolysis present in muscle and yeast appear to be present in epiphyseal cartilage. 2. Several of the phosphorylated intermediates of the cycle have been identified using chromatographic procedure.

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Further Studies on Relationship Between Human Serum Cholinesterase and Serum Albumin.* (19486)

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Of the two types of cholinesterase which have been described for the human, one, the specific or "true" cholinesterase, has received most of the emphasis in research because of its important role in the transmission of nerve impulses. The other, the cholinesterase of serum, the so-called "pseudo-cholinesterase", has received less attention because its specific function as an enzyme, as yet, has not been defined. Our own investigations, however, have made use of the latter enzyme as an indicator of albumin metabolism. We have reported(1) a close correlation between human serum cholinesterase and serum albumin levels, confirming the earlier work of Faber(2). In a series of 294 patients with a variety of diseases, we were able to demonstrate in all cases but those with albuminuria, that the serum albumin and the serum cholinesterase levels paralleled each other in a statistically significant manner.

A number of possibilities exist to explain the correlative relationship between the serum albumin and the serum cholinesterase, some of which are: 1) Albumin could act as a precursor to serum cholinesterase, and an albumin deficiency could cause the cholinesterase to decrease, as implied by Kunkel and Ward(3). 2) The decrease could be due primarily to a deficiency in protein intake, resulting in a general decrease of synthesis of all proteins both in serum and in the body organs. 3) The possibility exists that both the albumin and the serum cholinesterase are produced independently of each other, but by similar synthesizing mechanisms.

Methods. Patients were given concentrated normal human serum albumin, intravenously, containing 25 g of protein in 100 cc of neutral buffered diluent. *Protein determinations* were made by the biuret method of Weichselbaum(4) with two modifications: for the precipitation of globulins a solution of 26.8% sodium sulfate was used rather than 23% solution (giving lower albumin values than the customary Howe method which in-

* The albumin used in this study was provided by the National Blood Program of the American Red Cross.

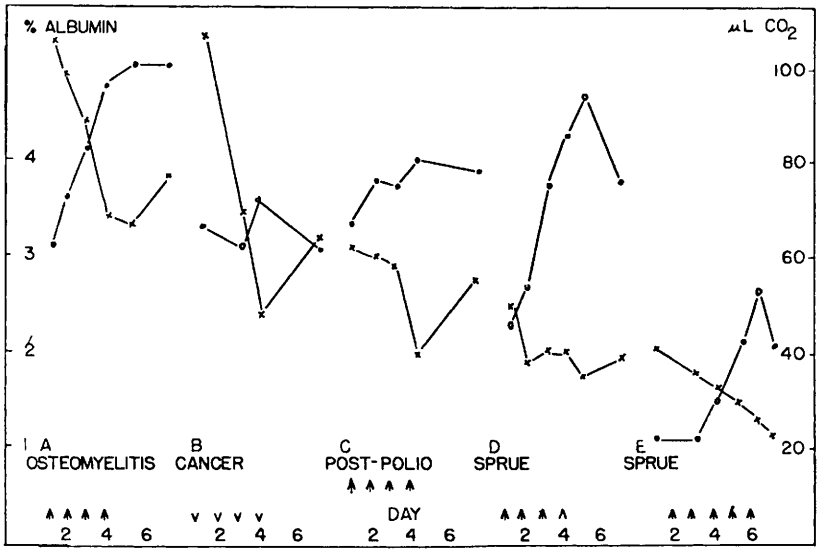

FIG. 1. Initial albumin cholinesterase relationships in five patients without overt liver damage. Arrows indicate administration of 75 g, and V's 25 g, of albumin. Cholinesterase x—x, albumin o—o.

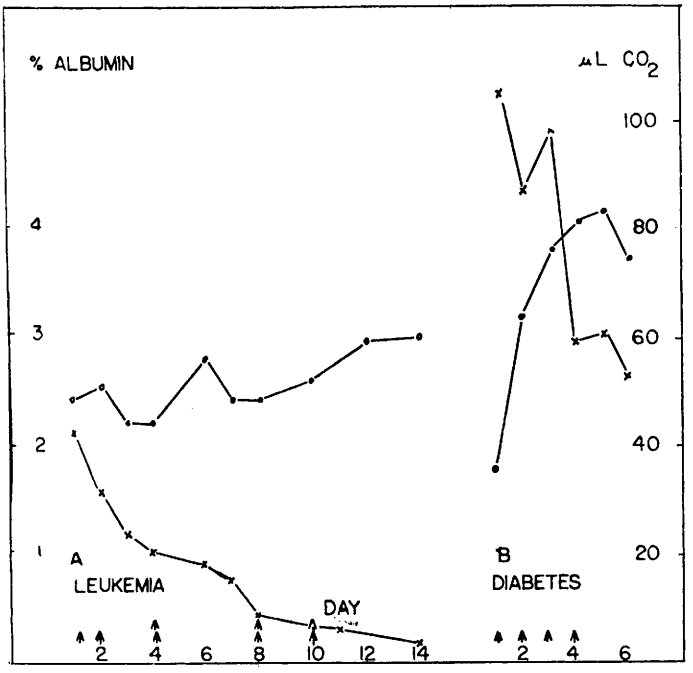

FIG. 2. The albumin cholinesterase relationship in two terminal cases. Arrows indicate administration of 75 g of albumin. Cholinesterase x—x, albumin o—o.

cludes the α-globulin with the albumin), and in spectrophotometric readings of the protein-biuret complex, allowance was made for interference by jaundiced or lipemic sera according to the procedure described by Keyser and Vaughn(5). *Serum cholinesterase activity* was determined by the Warburg technic as previously described by us(6). Results are expressed as μL carbon dioxide at 0°C and 760 mm mercury evolved in a 20-minute

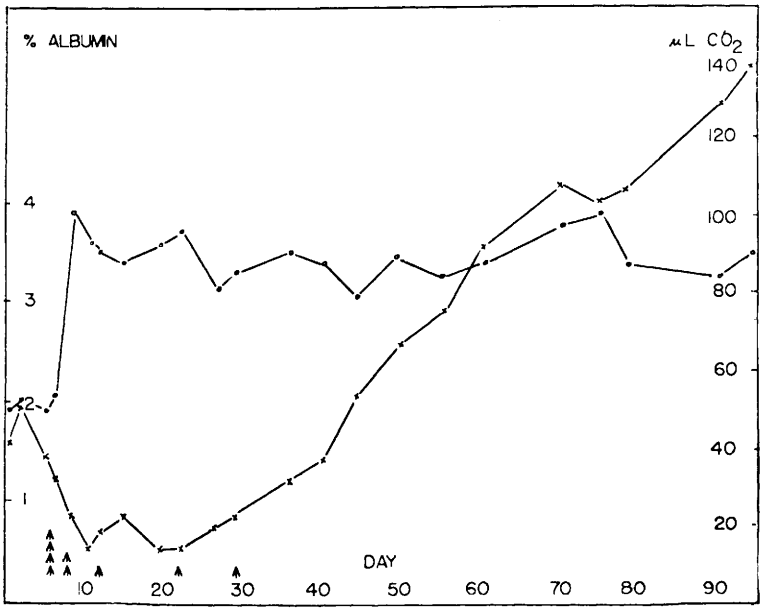

FIG. 3. Patient F. R. The relationship between albumin and cholinesterase in a case of infectious hepatitis. Arrows indicate administration of 75 g albumin. Cholinesterase x–x–x, albumin o–o–o.

period from a buffered bicarbonate solution. All values are corrected for non-enzymic hydrolysis and serum dilution factors. Both protein and cholinesterase values are adjusted to initial hematocrit values as a base line to allow for blood volume changes during albumin administration.

Results. Data are presented in graphic form showing serum albumin and serum cholinesterase values and amounts of administered albumin for a group of patients over varying periods of time. These observations are representative of the general responses observed in a larger series of patients.

Discussion. Of the possibilities suggested above to explain the correlation between the albumin and the serum cholinesterase levels, the first hypothesis, that albumin is a precursor for serum cholinesterase, is contradicted by the fact that, in spite of generally elevated serum albumin levels following intravenous administration of concentrated albumin to patients with initially low serum albumin values, there is neither a simultaneous nor a subsequent increase in the serum cholinesterase (Fig. 1, 2, and 3). Following the administration of concentrated albumin, serum albumin values remain constant or are elevated depending on the relative rate of utilization of administered albumin by the given patient. In most of our cases the administration of the albumin was followed by a rise in serum albumin levels. Despite the administration of from 100 to 300 g of concentrated albumin in these cases, there is a decrease in the serum cholinesterase level, which reaches a minimum on the fourth or fifth day following initiation of albumin administration. This decrease was also noted by Vorhaus(7), incidental to a study on cholinesterase in liver and biliary disease. If albumin were a precursor to serum cholinesterase, we would expect an increase in the cholinesterase values following the intravenous administration of the concentrated albumin; on the other hand, if the production of serum cholinesterase is independent of the amount of albumin present in the serum, we would expect no effect on the serum cholinesterase level following albumin administration. An explanation for the anomalous decrease in serum cholinesterase following albumin administration may be that administered serum albumin depressed the synthesizing systems

which produce both the albumin and the serum cholinesterase.

Possibilities 2 and 3 are not necessarily independent of each other. There is every likelihood that a deficiency of general protein precursors would necessarily restrict all protein synthesis, including albumin and serum cholinesterase. The emphasis in this paper, therefore, is on the third possibility, that albumin and serum cholinesterase are formed by the same or related synthesizing systems and there is good evidence that this synthesis occurs in the liver. The factors which influence albumin synthesis in the liver, whether actual liver dysfunction or a general shortage of protein precursors, cause both the serum albumin and serum cholinesterase values to vary together. Our own experience with patients with liver damage indicated that this enzyme is a valuable tool in the determination of the functional state of the liver.

If it is kept in mind that serum albumin and this enzyme are formed independently, but that the level of the enzyme indicates the ability of the liver to synthesize albumin, it becomes possible to utilize serial determinations of the enzyme as an index of liver albumin synthesis in the presence of administered albumin. Normally, such intravenously administered albumin would interfere with any determination of the ability of the liver to produce this protein by itself. In Patient A (Fig. 2) after intravenous administration of albumin, the albumin levels in the blood remain fairly constant, whereas serum cholinesterase continues to fall as liver failure progresses. Patient B (Fig. 2) is the only patient of the series presented here who shows an unusual initial relationship between the serum cholinesterase and albumin levels. This patient had a history of a long-standing nephritis and albuminuria, and illustrates the only condition in which a normal or high serum cholinesterase level is associated with a very low albumin level. It has been postulated that with albuminuria, the compensatory increase in albumin synthesis simultaneously increases the rate of production of serum cholinesterase(8). Following the administration of serum albumin, the initially high serum cholinesterase value fell. In both of these patients, the final low cholinesterase levels preceded death. In the case shown in Fig. 3, a patient with infectious hepatitis, the administration of albumin was indicated as a therapeutic procedure, but precluded the possibility of using serum protein levels as a guide to improvement. In other liver function tests, such as the cephalin flocculation, the icterus index, and the BSP test, there was no indication as to the course of the disease until the patient was quite obviously improved clinically. The cholinesterase determinations, however, showed that at first severe liver damage was present and the gradual but sustained return of the enzyme level to the normal range preceded obvious clinical recovery. These observations suggest that serum cholinesterase determinations are of prognostic value in the study of liver disease and that they are especially valuable where the administration of albumin intravenously would preclude serial protein determinations.

In summary, the cases presented in Fig. 2 and 3 demonstrate that serum cholinesterase levels vary with changes in the clinical conditions of the patients, independent of changes in serum albumin level as occasioned by intravenous serum albumin administration. This we take as evidence that serum albumin does not act as a precursor for serum cholinesterase. The initial unusual serum cholinesterase to albumin relationship in Fig. 2-B is common to cases of albuminuria. Further evidence that serum albumin is not a precursor for serum cholinesterase is presented in Fig. 1, where despite albumin administration, and following it, rather than an increase in serum cholinesterase levels, there is a depression.

Conclusions. The previously observed(1,2) correlation of serum albumin and serum cholinesterase values is probably due to the simultaneous production of these two proteins by related synthesizing mechanisms in the liver. Albumin does not act as a precursor of serum cholinesterase. Changes in the level of this enzyme reflect the changing ability of the liver to produce both serum cholinesterase and serum albumin. It is suggested that serum cholinesterase determinations are of prognostic value in liver disease and that cholinesterase determinations may be used as

an indication of albumin synthesis, particularly in the presence of administered albumin.

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Adrenal Ascorbic Acid Depletion by ACTH in Nephrectomized and in Subtotally Hepatectomized Rats.* (19487)

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The specificity of the adrenal ascorbic acid depletion test for pituitary adrenocorticotrophic hormone (ACTH) originally devised by Sayers *et al.*(1) is generally accepted, and the method has been widely used in assaying this hormone. In a recent communication it has been reported that when rat ACTH is injected into intact rats, large quantities are found in the kidneys(2). At the same time it cannot be detected in liver or urine(2). Absence from the liver could result from rapid hepatic inactivation. Its presence in the kidneys might reflect the importance of these organs in removing the hormone from general circulation. In view of the rapid disappearance of ACTH from the circulation(3) and the importance of the kidneys and liver in hormonal inactivation in general, it was felt that a study of the effect of these organs upon injected ACTH was warranted. This was tested by determining whether total nephrectomy or subtotal hepatectomy rendered a small quantity of ACTH, in itself sufficient to produce a moderate drop in adrenal ascorbic acid, more effective in this respect.

Materials and methods. Rats of the Holtzman strain were utilized in the present study. In a preliminary assay, designed to suggest a dosage meeting the requirements of the study to be made, a commercial ACTH powder (Armour, standardized against LA-1A) was obtained and from it 4 solutions in physiologic saline containing 4, 2, 1, and 0.5 μg/cc respectively were prepared. Thirteen female rats hypophysectomized 24 hours previously were divided into 4 groups. The left adrenal was removed in each animal under nembutal anesthesia, weighed on a Roller-Smith balance, placed in 6% trichloracetic acid, homogenized and the ascorbic acid determined by the method of Roe and Kuether(4). Each of the 4 concentrations of hormone was administered to a group of animals, each animal receiving 0.5 ml/100 g of the solution to be assayed by tail vein injection. One hour later the animals were sacrificed and the right adrenals re-

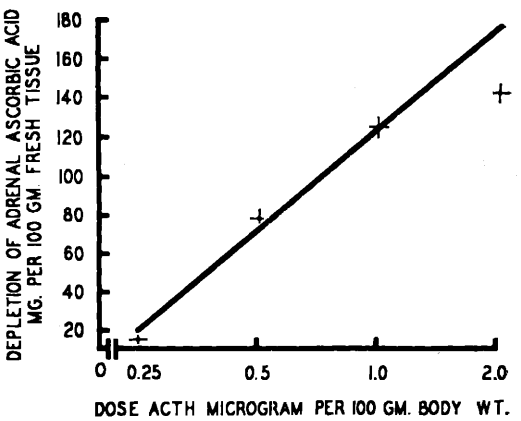

FIG. 1. Adrenal ascorbic acid depletion following intravenous ACTH.

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