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**Research Article**

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The ultrastructural examination of hepatic tissue from the five patients revealed increased amounts of glycogen. There was scarcity of endoplasmic reticulum. There was intercellular glycogen in continuity with the glycogen of the hepatocytes through breaks in their circumference. Lipid droplets with lucid areas in the form of needles and plates contained aggregates of glycogen. There were numerous lysosomes, some containing glycogen. Large vacuoles filled with glycogen and surrounded by a membrane were seen occasionally. The vacuoles might reflect the lysosomal pathway of glycogen degradation, since there was apparent fusion of such autophagic vacuoles with small vesicles resembling primary lysosomes.



# Deficient Activity of Dephosphophosphorylase Kinase and Accumulation of Glycogen in the Liver

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ABSTRACT Low activity of phosphorylase and increased concentration of glycogen were found in liver tissue from five children with asymptomatic hepatomegaly. In vitro activation of liver phosphorylase in these patients occurred at the rate of 10% or less of normal. Elimination of the defect by the addition of kinase that activates phosphorylase demonstrated the integrity of the phosphorylase enzyme and the deficient activity of dephophophosphorylase kinase.

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The ultrastructural examination of hepatic tissue from the five patients revealed increased amounts of glycogen. There was scarcity of endoplasmic reticulum. There was intercellular glycogen in continuity with the glycogen of the hepatocytes through breaks in their circumference. Lipid droplets with lucid areas in the form of needles and plates contained aggregates of glycogen. There were numerous lysosomes, some containing glycogen. Large vacuoles filled with glycogen and surrounded by a membrane were seen occasionally. The vacuoles might reflect the lysosomal pathway of glycogen degradation, since there was apparent fusion of such autophagic vacuoles with small vesicles resembling primary lysosomes.

## INTRODUCTION

Among the children with hepatomegaly seen at the Clinical Research Center during the past 4 yr, there were five patients with a similar clinical, biochemical, and histological pattern. Clinically, the liver was greatly enlarged but no other abnormality was present. Biochemically, the phosphorylase activity of the liver biopsy specimen was low and the glycogen concentration was increased. Histologically, excessive glycogen was present in the hepatocytes.

Evaluation of the low phosphorylase activity posed a special problem. Total phosphorylase is the sum of the active and of the inactive form that are interconverted enzymatically (1). The usual measurement of hepatic phosphorylase ("phosphorylase activity") demonstrates only the fraction of the total phosphorylase that is in the active form. Thus, low activity may result from deficiencies of the phosphorylase enzyme itself, from deficiencies of the enzymes in the phosphorylase-activating system, or from deficiencies of the control over the degree of phosphorylase activation.

To locate the defect in our patients, a system for in vitro activation of phosphorylase was developed. The total phosphorylase activity in "normal" human liver was measured utilizing the same system.

## METHODS

*Description of the patients.* The five patients with their age at the time of the diagnostic liver biopsy were: a girl, R. E. (4 yr); an unrelated boy, S. D. (1¾ yr); twin girls, C. H. and L. H., (6½ yr), and their younger brother H. H. (3½ yr). Erythrocyte typing and dermatoglyphics of C. H. and L. H. supported the impression that they are identical twins. There was no demonstrable consanguinity in the three families. The six parents did not have hepatomegaly. Siblings of the patients had no hepatic enlargement. They were: four sisters of R. E.; one sister of S. D.; two brothers and four sisters of C. H., L. H., and H. H. In each case the parents stated that the patient's abdomen had been protuberant since birth. There were no other complaints, in particular no jaundice, no mental retardation, and no symptoms suggesting hypoglycemia.

On the initial hospital admission, the liver edge was 10 cm below the costal margin in the mid-clavicular line in R. E.,

A preliminary report of the first patient (R. E.) was published in 1966 *Science.* **153:** 1534.

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12 cm in S. D., 8 cm in C. H., 10 cm in L. H., and 9 cm in H. H. On the anthropometric growth chart, the body length for the respective age was: R. E. and S. D. in the 10th percentile; C. H., L. H., and H. H. below the 3rd percentile. The body weight for the respective age was: R. E. in the 35th percentile; S. D. in the 25th percentile; C. H., L. H., and H. H. in the 10th percentile. There were no other abnormal findings on physical examination. In none of the children was there any clinical or biochemical evidence for hypoglycemia during the 7 months of their combined hospitalizations. Normal values were obtained on the following determinations in the serum of the five patients: $CO_2$, Cl, BUN, Na, K, Ca, P, alkaline phosphatase, glucose, bilirubin direct and indirect, total protein, albumin, globulin, and lactic dehydrogenase. Abnormal values were obtained on the following determinations in the serum: cholesterol, glutamic-oxaloacetic transaminase[1] glutamic-pyruvic transaminase,[2] aldolase,[3] creatine phosphokinase,[4] total lipids, and uric acid (Table I). Hemograms and urine analyses were normal. There was no acetone in 37 urine specimens from the five patients. The following additional examinations were done on one or more patients with normal results: bone marrow examination, sweat chloride concentration, intravenous pyelogram, electrocardiogram, examination of the chest and of the osseous maturation by X-ray, concentration of amino acids in the urine, and 24-hr urinary excretion of free epinephrine and of free norepinephrine.

In all patients, the intravenous administration of water-soluble crystalline glucagon in the test dose of 0.7 mg/m$^2$ of body surface produced a rise in blood sugar concentration that was within the 95% confidence limits for normal children established by Vassella (2).

One patient (R. E.) received daily intramuscular injections of zinc glucagon.[5] Three such attempts to mobilize hepatic glycogen and reduce liver size were made for periods of 20, 7, and 2 wk, using daily doses of glucagon of from 1.4 to 6 mg (Fig. 1). During each treatment period, the size of the liver decreased markedly and promptly; but the return to pretreatment size was just as prompt after glucagon administration was stopped. There were no other detectable effects of the drug.

At present, the five patients are doing well at home without therapy.

*Liver and muscle biopsies.* Liver biopsies were done with the Menghini needle (3). Muscle biopsies were done with the Vim Silverman needle with Franklin modification. Liver

[1] Determination of serum glutamic oxalacetic transaminase, Dade Reagents, Inc., Miami, Fla.

[2] Determination of serum glutamic pyruvic transaminase, Dade Reagents, Inc., Miami, Fla.

[3] Biochemica test combination, Aldolase-Test, C. F. Boehringer und Soehne GmbH, Mannheim.

[4] Biochemica test combination, CPK-Test, C. F. Boehringer und Soehne GmbH, Mannheim.

[5] Dr. John A. Galloway, Medical Research Division, Eli Lilly and Company, Indianapolis, Ind., generously supplied long-acting zinc glucagon before the investigational status and the production of the drug were terminated.

TABLE I

*"Abnormal" Serum Chemistries*

| | R. E. | S. D. | C. H. | L. H. | H. H. | Normal |
|---|---|---|---|---|---|---|
| Cholesterol, *mg/100 ml* | | | | | | |
| Mean | 288 | 240 | | 180 | | |
| Range | 263–362 (14)* | 264; 216 (2) | 220 (1) | 200; 160 (2) | 200 (1) | 150–250 |
| SGO-T, *units* | | | | | | |
| Mean | 85 | 322 | | 99 | | |
| Range | 24–143 (13) | 75–490 (3) | 19 (1) | 172; 27 (2) | 41 (1) | 8–40 |
| SGP-T, *units* | | | | | | |
| Mean | 102 | 101 | | 110 | | |
| Range | 30–225 (12) | 53–170 (3) | 15 (1) | 210; 11 (2) | 25 (1) | 5–30 |
| Aldolase, *units* | | | | | | |
| Mean | 7.7 | | | | | |
| Range | 3.5 – 14.5 (3) | 12.3 (1) | Not done | Not done | Not done | 0.9–2.5 |
| CPK, *units* | | | | | | |
| Mean | 1.8 | | | | | |
| Range | 2.5; 1.1 (2) | 0.8 (1) | Not done | Not done | Not done | 0–1 |
| Total lipids, *mg/100 ml* | | | | | | |
| Mean | 964 | 785 | | | | |
| Range | 784–1894 (13) | 782; 789 (2) | 698 (1) | 315 (1) | 731 (1) | 500–750 |
| Uric acid, *mg/100 ml* | | | | | | |
| Mean | | 6.3 | | 7.0 | | |
| Range | 5.6; 4.6 (2) | 6.8; 5.8 (2) | 6.4 (1) | 7.3; 6.8 (2) | 5.7 (1) | 3.0–5.5 |

* Figures in parentheses indicate number of separate determinations.

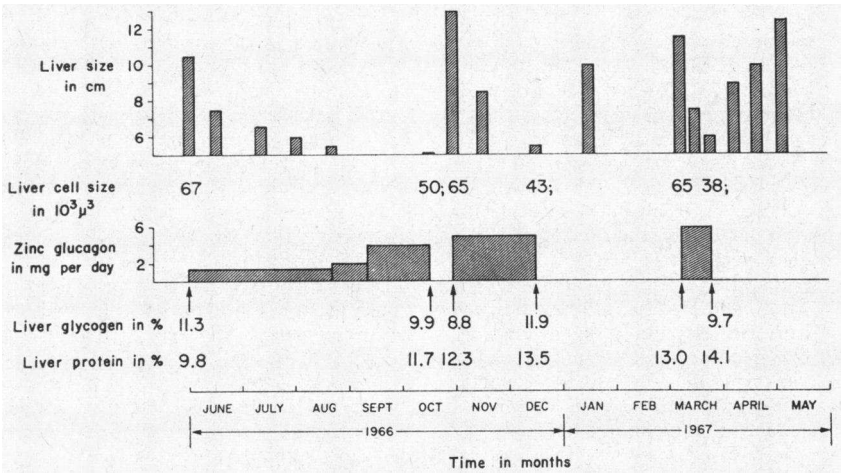


FIGURE 1 Administration of zinc glucagon to patient R. E. Arrows (↑) indicate liver biopsy.

tissue for control experiments was available from nine children with a variety of other conditions that required biopsy of the liver.

*Biochemical assays.* The activity of liver phosphorylase was determined according to Sutherland (4). The details of our method have been published (5). For the diagnostic biopsies (Table II), phosphorylase activity was expressed as $\mu$moles of phosphate liberated from glucose-1-phosphate/g of liver tissue per min. For the in vitro activation of phosphorylase (Tables III and IV), activity was expressed as $\mu$moles of phosphate/mmole of nitrogen of liver tissue per min. This could be converted approximately into $\mu$moles of phosphate/g of liver per min by multiplication with the factor of 1.71 that was based on the atomic weight of nitrogen (14), the approximate protein concentration of liver (15%), and the conversion factor of nitrogen into protein (6.25) (6). The liver protein concentration of 15%, SD 3.3%, was based on nitrogen determinations in 47 liver homogenates from 12 individuals. Nitrogen was determined by the micro-Kjeldahl method (7).

Muscle phosphorylase b kinase was prepared from rabbit muscle (8). The preparation converted 60,000 units of crystalline rabbit muscle phosphorylase b to phosphorylase a/min per mg of protein.

In vitro activation of phosphorylase was performed as follows. Immediately after the biopsy, the liver specimen was homogenized with a glass hand-homogenizer in ice-cold 0.04 M Tris-sodium $\beta$-glycerophosphate buffer, pH 7.8. The concentration of the homogenate was 80 mg of liver tissue/ml. Initial phosphorylase activity of the homogenate was determined at once. The value was in good agreement with the phosphorylase activity of a separate portion of the biopsy specimen homogenized in the usual manner, i.e., with the addition of sodium fluoride to inhibit the action of phosphorylase phosphatase (5). The liver tissue homogenized in Tris-sodium $\beta$-glycerophosphate buffer was incubated in the water bath at 37°C for 15 min (hereafter designated as preincubation) when 0-time activity of phosphorylase was determined in an aliquot of the homogenate. The homogenate was then distributed into five tubes, such that in each tube the concentration of liver tissue was 20 mg/ml in a reaction mixture that, with proper 0-time additions, had been adjusted to the final concentration: $10^{-3}$ M ATP, $10^{-6}$ M 3',5'-AMP, $5 \times 10^{-2}$ M NaF, $5 \times 10^{-3}$ M $MgCl_2$, and $2 \times 10^{-2}$ M Tris-sodium $\beta$-glycerophosphate buffer, pH 7.8. Further additions to the five tubes were: none to tube one; 20 mg of liver tissue/ml from another of the five patients to tube two; 20 mg of liver tissue/ml from a control hepatic biopsy specimen to tube three; 6 $\mu$g of phosphorylase b kinase from rabbit muscle to tube four; and 5 mg of skeletal muscle tissue from a patient with congenital absence of muscle phosphorylase (McArdle's syndrome) to tube five. Tube five was further homogenized for 30 sec, since the muscle had been

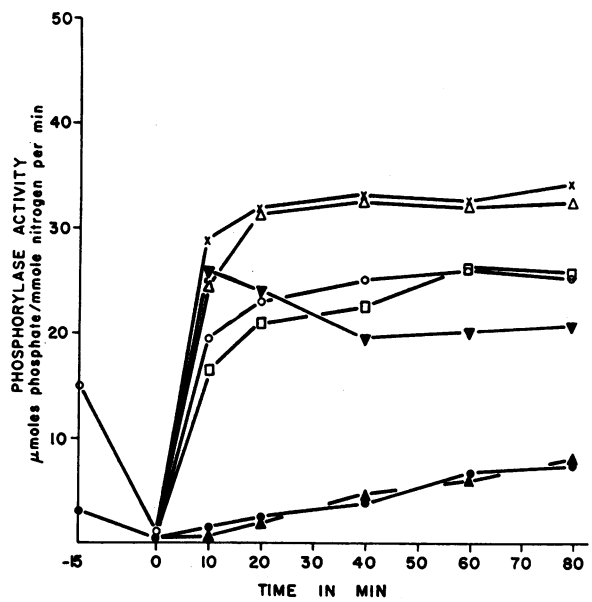


FIGURE 2 Mean activity for each type of homogenate are identified as follows: ——●——, patient liver, no other tissue; ——▲——, patient liver and patient liver; ——▼——, patient liver and McArdle muscle; ——□——, patient liver and control liver; ——×——, patient liver and rabbit kinase; ——○——, control liver, no other tissue; and ——△——, control liver and rabbit kinase.

TABLE II

*Diagnostic Liver Biopsies—Biochemical Data*

| Cases | Phosphorylase | Glycogen concentration | α-Glucosidase | Glucose-6-phosphatase | Amylo-1,6-glucosidase: Glucose-$^{14}$C incorporation into -1,6- branch points of glycogen. Qualitative assay |
|---|---|---|---|---|---|
| | *μmoles phcsphate/ g per min* | *% of weight of wet tissue* | *μmoles glucose/ g per min* | *μmoles phosphate/ g per min* | |
| Normal | 25.1 ±6.5* (42)‡ | 2–6 | 0.258 ±0.093* (43) | 4.7 ±1.9* (35) | |
| Case I (R. E.) | | | | | |
| Mean | 2.3 | 9.9 | 0.155 (1) | 3.3 (1) | Normal |
| Range | 0–9.7 (7) | 7.7–14.0 (7) | | | |
| Case II (S. D.) | | | | | |
| Mean | 3.0 | 10.2 | 0.123 (1) | 2.4 (1) | Normal |
| Range | 1.3–6.7 (5) | 7.9–11.4 (4) | | | |
| Case III (L. H.) | | | | | |
| Mean | 6.2 (1) | 8.4 | 0.152 (1) | 4.6 (1) | Not done |
| Range | | 7.2; 9.5 (2) | | | |
| Case IV (C. H.) | 7.3 (1) | 9.2 (1) | Not done | 3.7 (1) | Not done |
| Case V (H. H.) | 2.4 (1) | 9.8 (1) | Not done | 4.7 (1) | Not done |

* Mean activity ±1 SD.

‡ Figures in parentheses indicate: in the case of the normals, the number of different individuals; and in the case of the patients, the number of different biopsy specimens.

added as solid tissue. The tissue additions to tubes two and three were made out of homogenates of hepatic biopsy specimens that were obtained and assayed at the same time as the patient's biopsy specimen. The final volume of the reaction mixture in each tube was 0.5 ml.

After completion of the 0-time additions, phosphorylase activity was determined on aliquots removed after 10, 20, 40, 60, and 80 min of incubation in the water bath at 37°C. The phosphorylase impurities of the phosphorylase b kinase preparation that was added to tube four did not contribute any activity to the final phosphorylase determination of the experiment, as shown by the incubation of control tubes that contained kinase and the 0-time additions but no liver tissue.

While this study was in progress, it was found in separate experiments that the omission of 3′,5′-AMP from all reaction mixtures did not alter the pattern of phosphorylase activation. The addition of ATP in the concentration of $10^{-3}$ M was essential. Activation was not obtained when ATP was present in the concentration of either $10^{-2}$ or $10^{-4}$ M.

The activity of glucose-6-phosphatase was measured with the reaction mixture of Cori (9). Incubation was in the water bath at 37°C and activity was expressed as μmoles of phosphate liberated from glucose-6-phosphate/g of liver tissue per min.

Amylo-1.6-glucosidase was measured according to Hers (10) and as described previously (11). Acid α-glucosidase was measured according to Hers with glycogen as substrate (12).

The concentration of glycogen was determined in 5–10 mg of the biopsy specimen. The aliquot was added to 0.1 ml of 30% NaOH and kept in a boiling water bath for 90 min. With 6 N HCl, the tissue digest was adjusted to 2 N HCl and hydrolyzed for 3.5 hr in a boiling water bath. The hydrolysate was neutralized and was made up to 10 ml with water. Further dilution (usually 1:10) was necessary before analysis for glucose with the glucose-oxidase method (13).

In separate experiments, this method of glycogen determination was compared with that of Pflüger (14). The extent of the difference between the two methods was not important biologically.

TABLE III

*In Vitro Activation of Hepatic Phosphorylase: "Normal" Controls*

| Source of added kinase | Periods of incubation (*min*) | | | | | | |
|---|---|---|---|---|---|---|---|
| | −15 | 0 | 10 | 20 | 40 | 60 | 80 |
| | *μmoles phosphate/mmole of N liver tissue per min* | | | | | | |
| None | | | | | | | |
| Mean | 15.0 | 0.9 | 19.7 | 23.1 | 24.8 | 25.9 | 25.2 |
| SD* | 4.5 | 0.5 | 4.8 | 5.0 | 5.1 | 5.6 | 6.1 |
| *N*‡ | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Tests§ | 22 | 22 | 22 | 22 | 22 | 22 | 21 |
| Rabbit muscle | | | | | | | |
| Mean | | | 29.2 | 32.0 | 33.0 | 32.3 | 34.0 |
| SD | | | 5.0 | 6.5 | 7.9 | 6.6 | 6.8 |
| *N* | | | 9 | 9 | 9 | 9 | 9 |
| Tests | | | 19 | 19 | 19 | 19 | 19 |

* SD, standard deviation.

‡ *N*, number of different individuals.

§ Tests, number of different activation experiments.

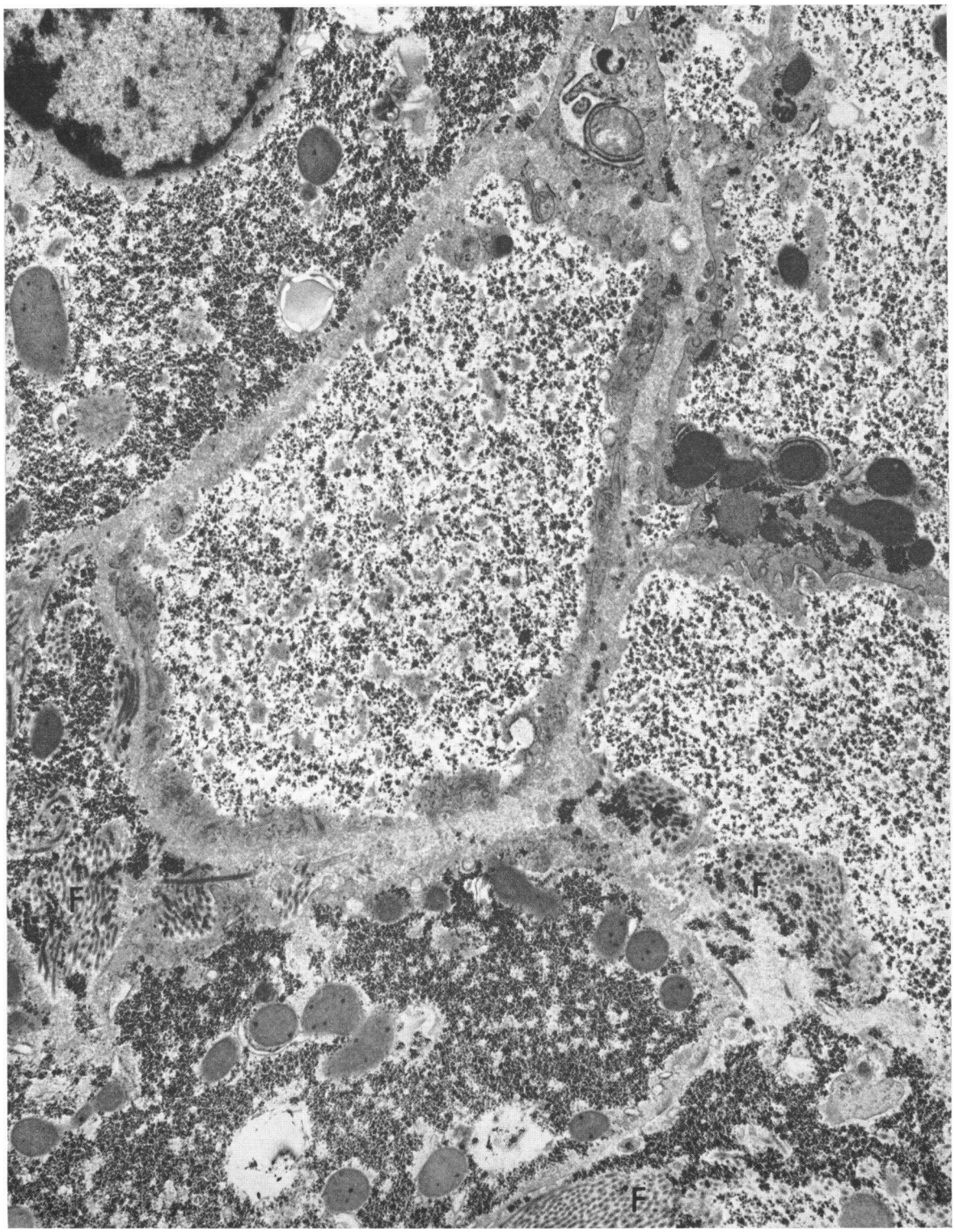


FIGURE 3 Liver: patient S. D. Adjacent cells show abrupt changes in the concentration of glycogen. There is scarcity of endoplasmic reticulum. Horizontal and oblique sections of collagen fibers are seen in intercellular spaces (*F*). Approximately × 13,000.

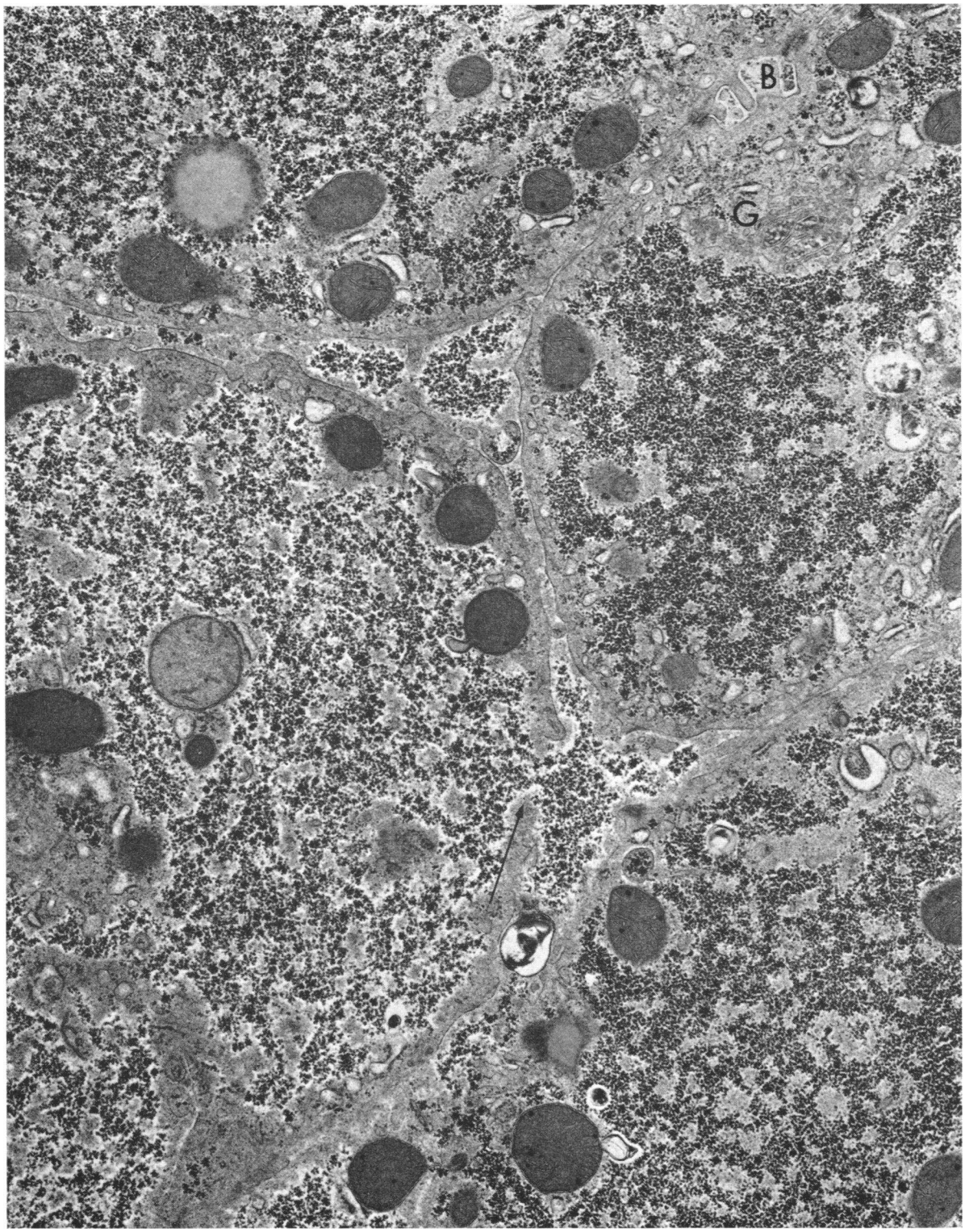


FIGURE 4 Liver: patient S. D. Glycogen is abundant and membranes of the endoplasmic reticulum are scarce. Glycogen of a hepatocyte is in continuity with intercellular glycogen through a break in the cellular circumference indicated by the arrow. *G.* Golgi apparatus; *B.* bile canaliculus. Approximately × 17,000.

TABLE IV

*In Vitro Activation of Hepatic Phosphorylase: Patients*

| Source of added kinase | Periods of incubation (*min*) | | | | | | |
|---|---|---|---|---|---|---|---|
| | −15 | 0 | 10 | 20 | 40 | 60 | 80 |
| | *μmoles phosphate/mmole of N in liver tissue per min* | | | | | | |
| None | | | | | | | |
| Mean | 3.1 | 0.6 | 1.4 | 2.3 | 3.9 | 6.6 | 7.7 |
| SD* | 1.5 | 0.4 | 1.0 | 1.5 | 1.4 | 3.1 | 3.7 |
| *N*‡ | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Tests§ | 14 | 14 | 14 | 14 | 14 | 14 | 14 |
| Rabbit muscle | | | | | | | |
| Mean | | | 24.6 | 31.3 | 32.3 | 32.0 | 32.2 |
| SD | | | 5.9 | 8.8 | 7.1 | 7.0 | 9.3 |
| *N* | | | 5 | 5 | 5 | 5 | 5 |
| Tests | | | 14 | 14 | 14 | 14 | 14 |
| Control liver | | | | | | | |
| Mean | | | 16.0 | 21.0 | 22.4 | 25.8 | 25.4 |
| SD | | | 7.3 | 7.8 | 6.7 | 7.7 | 5.7 |
| *N* | | | 4 | 4 | 4 | 4 | 4 |
| Tests | | | 9 | 9 | 9 | 9 | 9 |
| McArdle muscle | | | | | | | |
| Mean | | | 26.2 | 24.0 | 19.4 | 20.1 | 20.7 |
| SD | | | 6.8 | 10.7 | 6.1 | 6.3 | 6.9 |
| *N* | | | 3 | 3 | 3 | 3 | 3 |
| Tests | | | 4 | 4 | 4 | 4 | 4 |
| Patient liver | | | | | | | |
| Mean | | | 0.4 | 2.2 | 4.3 | 6.0 | 8.1 |
| *N* | | | 2 | 2 | 1 | 2 | 2 |
| Tests | | | 2 | 2 | 1 | 2 | 2 |

*, ‡, §: see footnotes to Table III.

*Electron microscopic examination.* The tissue specimens were immersed immediately after the biopsy in cold (0°–5°C) buffered 3% glutaraldehyde (pH 7.3; 0.1 M phosphate buffer), cut into small pieces, kept in the refrigerator for 24 hr, and washed overnight in cold buffer. Postfixation was in cold (0°–5°C) buffered 1% osmium tetroxide (pH 7.3; 0.1 M phosphate buffer) for 90 min. After dehydration in graded ethanol, the tissue was embeded in Epon 812. Thin sections were cut with a diamond knife on a Reichert OMU 2 ultramicrotome, stained on uncoated grids with uranyl acetate (5 min) followed by lead citrate (5 min), and examined in a Philips 300 electron microscope.

*Assessment of cell size.* The average hepatic cell size in biopsy specimens obtained before and after zinc glucagon treatment was determined as described by Cussen for smooth muscle (15). Allowance was made for multinuclear hepatocytes.

## RESULTS

*Initial biochemical analysis.* The results of the diagnostic liver biopsies are listed in Table II. In the patients, liver phosphorylase activity was decreased and liver glycogen concentration was increased. Enzymatic activities defective in other types of glycogen storage disease were normal in liver and in skeletal muscle, as was the concentration of glycogen in skeletal muscle. In particular, there was no detectable deficiency of muscle phosphorylase activity. Thus, the following comments pertain to the activity of liver phosphorylase.

*In vitro activation of phosphorylase.* The results are listed in Table III for the controls and in Table IV for the patients whereas the mean values for both groups are plotted in Fig. 2.

(*a*) Activation of phosphorylase in homogenate of liver from controls: the initial activity of control phosphorylase (i.e., the activity at the time of the biopsy) was lost during the 15 min of preincubation. With the 0-time addition of cofactors and of sodium fluoride, the activity was restored rapidly and reached a plateau at a value higher than that of the initial activity. On the average, the initial activity was 60% of the final total activity. When phosphorylase kinase from rabbit muscle

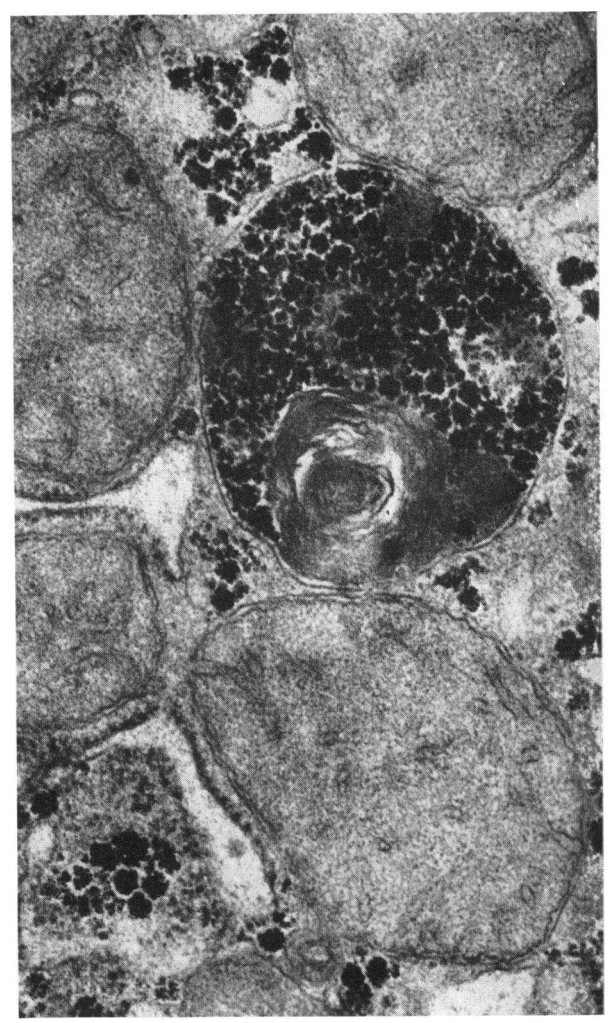

FIGURE 5 Liver: patient R. E. This autophagic vacuole with glycogen and a whorl of membranes is comparable in size to mitochondria. Approximately × 50,000.

was added to normal control liver, the total activity was higher than without added phosphorylase kinase.

(*b*) Activation of phosphorylase in the homogenate of liver from patients: the initial phosphorylase activity of the patients was markedly lower than that of the controls. After the 15 min of preincubation, the 0-time activity of the patients was indistinguishable from that of the controls. The 0-time additions led to a reactivation of the patients' phosphorylase at 10% or less of the rate that was obtained with the controls. The deficiency was rectified by the addition of phosphorylase kinase, either in the form of purified phosphorylase kinase from rabbit muscle, of skeletal muscle homogenate from an individual with McArdle's syndrome, or of control liver homogenate. In contrast, the rectifying effect was not obtained by the addition of liver homogenate from another patient.

(*c*) Statistical evaluation: for each time of sampling during the activation, the means of phosphorylase activity in the various homogenates were compared using the *t* test (16). The mean activity of patients differed significantly either from the normals ($P < 0.01$) or from the patients with added muscle phosphorylase kinase ($P < 0.01$), or from the patients with added normal liver ($P < 0.01$ except for 80 min of incubation where $P < 0.05$). There was no significant difference between the normals and the patients fortified with normal liver, or between the normals and the patients after both these homogenates had been fortified with purified muscle phosphorylase kinase. There was a statistical difference between the mean activity of the normals and of the normals with added kinase ($P < 0.01$ except for 40 and 60 min of incubation where $P < 0.05$).

*Electron microscopic analysis.* No unusual features were detected in the fine structure of the skeletal muscle.

In the hepatocytes, glycogen was increased; the amount varied considerably from one cell to another. Frequently, glycogen was observed in intercellular spaces. Adjacent cells sometimes had breaks in their circumference that were filled with glycogen particles. Continuity between the intracellular and the intercellular glycogen resulted. Frequently, collagen fibers mingled with the glycogen in the intercellular spaces (Figs. 3 and 4). Lysosomes were present usually adjacent to the cellular borders and to the bile canaliculi. Some vacuoles were partly filled with glycogen. They attained the size of mitochondria; rarely were they considerably larger, resembling the autophagic vacuoles observed in type II glycogenosis (Figs. 5 and 6).

There were a moderate number of lipofuscin bodies. Many lipid droplets ranging in size from that of mitochondria to the size of nuclei were present. These droplets varied in electron density from dark (almost black) to lucid (almost colorless). Some droplets had lucid areas in the shape of needles or plates. Some droplets contained aggregates of glycogen (Fig. 7).

## DISCUSSION

The phosphorylase enzyme exists in an active form (phosphophosphorylase) and in an inactive form (dephosphophosphorylase) that together comprise the amount of total phosphorylase. Phosphophosphorylase phosphatase catalyzes the conversion of the active to the inactive form. Dephosphophosphorylase kinase catalyzes the conversion of the inactive to the active form, a reaction that requires the presence of ATP and of bivalent ions. Dephosphophosphorylase kinase is activated in the presence of cyclic 3′,5′-AMP that results from the action of adenyl cyclase on ATP. Adenyl cyclase in turn is stimulated by hormones (17).

The usual phosphorylase assay measures the activity of phosphophosphorylase but not that of dephosphophosphorylase. Knowledge of the total activity of human liver phosphorylase and how it is attained was necessary in order to evaluate the low activity of the enzyme in our patients. Hers reported three cases of low liver phosphorylase activity. Incubation of hepatic tissue with ATP, $MgCl_2$ and 3′,5′-AMP did not increase activity (10). Such cases have been recorded as "hepatophosphorylase deficiency (type VI) glycogenosis" (18, 19). In our experience, kinase activity is lost in stored frozen tissue. At room temperature, phosphorylase is deactivated in biopsy material (5). Without information as to how the liver tissue was obtained, stored, and assayed, the reported cases of low phosphorylase activity cannot be interpreted conclusively.

The system for in vitro activation of phosphorylase in human liver was established using similar conditions as described by Sutherland and Wosilait (1). During the 15 min of preincubation, phosphophosphorylase phosphatase produced complete deactivation in liver homogenates of both controls and patients. In the controls, the 0-time additions initiated rapid reactivation by dephosphophosphorylase kinase. The reaction proceeded to a plateau assumed to reflect the amount of total phosphorylase.

In the patients, the initial activity of hepatic phosphorylase was always low. However, fluctuations occurred in successive specimens obtained from the same liver. For instance, in seven biopsies of the patient R. E. done over a period of 4 yr, the variation was from 0 to 9.7 μmoles P/g per min (5.8 μmoles P/mmole N per min) with the mean of 2.3 μmoles P/g per min (1.4 μmoles P/mmole N per min).

This considerable variation made it unlikely that deficiency of the phosphorylase enzyme would account for the low activity. Indeed, the total activity of hepatic

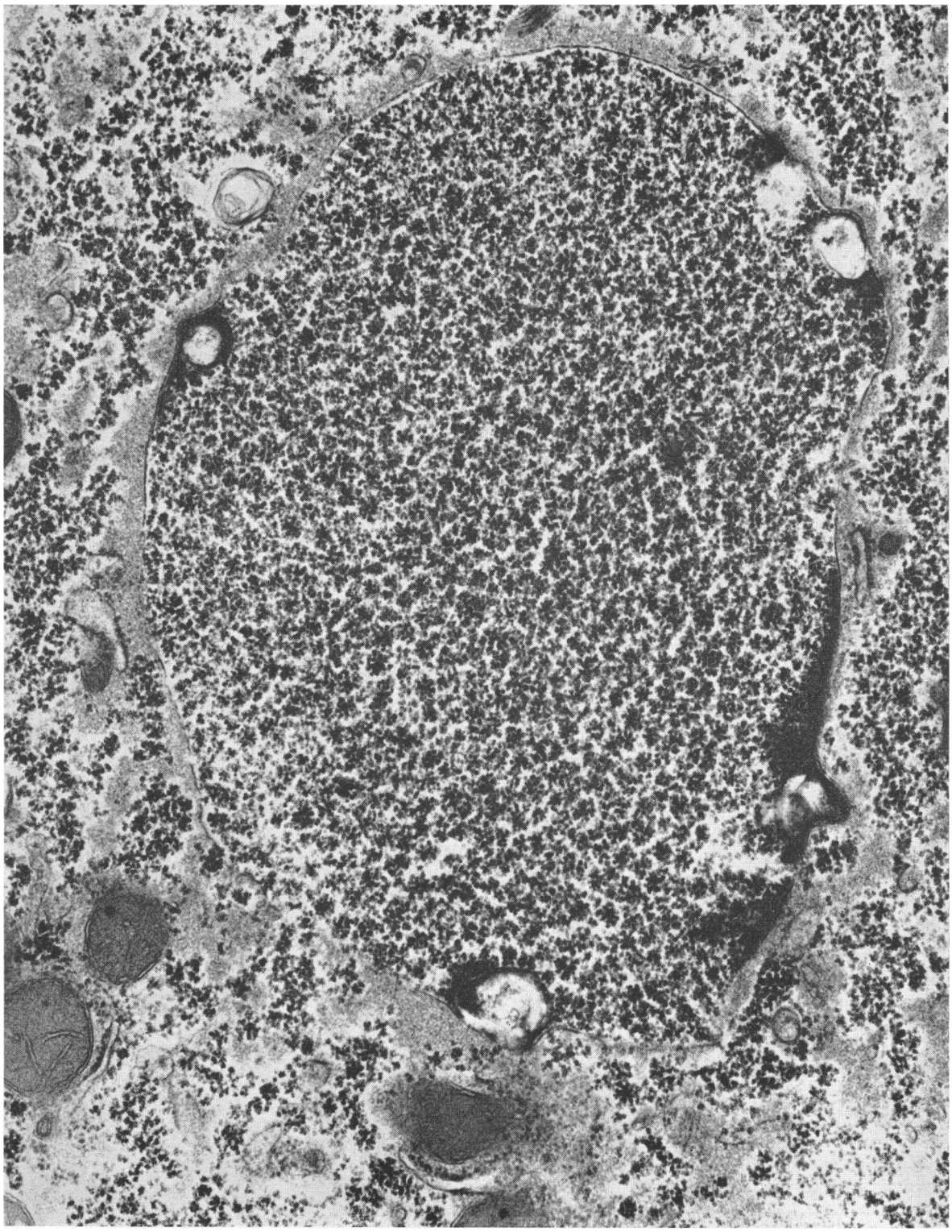

FIGURE 6

phosphorylase of the patients could not be distinguished from the total activity of the normal liver once the homogenates of the patients were fortified by phosphorylase kinase from one of the three different sources. These observations led us to conclude that the hepatocytes of the five patients were deficient in the activity of dephosphophosphorylase kinase.

Although kinase is not activated by ATP, $MgCl_2$, and 3',5'-AMP in homogenates of stored frozen liver, the additional inclusion of rabbit muscle phosphorylase kinase in such homogenates will still produce phosphorylase activation. Frozen liver can thus be analyzed for deficiency of phosphorylase but not of dephosphophosphorylase kinase.

As a result of kinase deficiency, no increase in the concentration of blood glucose after the intravenous administration of crystalline glucagon would be anticipated. However, the intravenous glucagon tolerance test was normal in all five patients. We cannot explain the discrepancy.

One patient received long-acting zinc glucagon to determine whether the drug might mobilize glycogen by maintaining the residual kinase in the active state until near normal phosphorylase activity was achieved. During the three treatment periods, both the hepatomegaly and the average size of the liver cell decreased, the latter by approximately 30%. If the decrease was accomplished by selective mobilization of glycogen and of its water of deposition (about 2 ml/g of glycogen (20)), protein concentration of the remaining liver would increase from 10 to 14% and glycogen concentration would drop from 10 to 0%. Whereas such an increase in protein concentration might escape detection, the drop in glycogen concentration would be shown by our method. Neither the concentration of glycogen nor of protein changed appreciably during glucagon administration. This observation implies proportional but not selective loss of glycogen.

The prognosis of the condition is not known. None of our patients showed biochemical or clinical evidence of hypoglycemia. They are of short stature, three of them being below the 3rd percentile. Their mental development is normal and they are doing well at home on no therapy.

However, the ultrastructural examination of the hepatic specimens showed a moderate but definite degree of fibrosis that we have not observed in hepatic specimens from two children with type I glycogenosis. In both varieties of glycogen storage disease, the hepatocytes appeared engorged with glycogen. The abrupt changes in the glycogen concentration of adjacent cells and the breaks in the cellular circumference seen regularly in these patients were not present in children with type I glycogenosis, nor were the abundant intercellular glycogen and the lipid droplets with glycogen inclusions.

Membrane-surrounded vacuoles filled with glycogen occur much more frequently in the liver of type II glycogenosis, but without the peripheral small vesicles demonstrated in Fig. 6. The small vesicles resemble primary lysosomes. Their apparent fusion with an autophagic vacuole might represent the lysosomal pathway of glycogen degradation, i.e., an alternative pathway to the degradation by phosphorylase. This interpretation seems difficult to reconcile with the rarity of large autophagic vacuoles in the present patients and with the complete absence of such vacuoles in type I glycogenosis where the amount of glycogen is just as excessive.

For a previous comparative study of four different types of glycogen storage disease, excessive accumulation of glycogen in the liver with deficient activity of hepatic dephosphophosphorylase kinase was abbreviated as type IX glycogenosis (21).

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FIGURE 6 Liver: patient S. D. Under the surrounding membrane of a large autophagic vacuole filled with glycogen there are several clear vesicles that might represent primary lysosomes. Their hydrolytic enzymes would initiate the degradation of the vacuolar content as perhaps indicated in the picture by the dark zones around the vesicles. Similar vacuoles but without the peripheral clear vesicles occur much more frequently in liver specimens of type II glycogenosis. Approximately × 26,000.

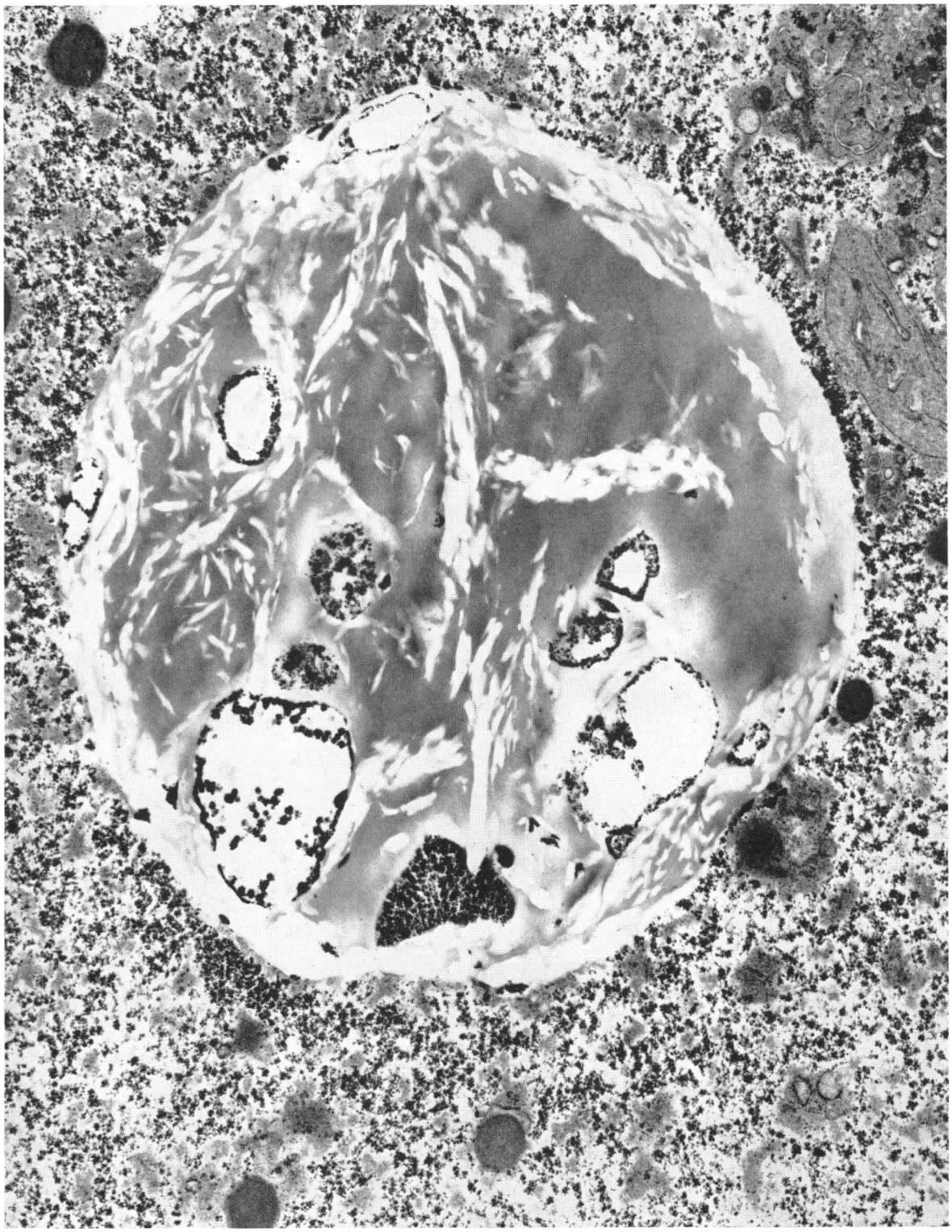

FIGURE 7 Liver: patient S. D. Such lipid droplets are seen regularly that contain glycogen inclusions and lucid areas in the form of needles and plates. Approximately × 17,000.

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