Abnormal Fatty Acid Metabolism in Peripheral Nerves of Patients with Pernicious Anemia

EUGENE P. FRANKEL

From the Evelyn L. Overtor Hematology-Oncology Research Laboratory, Department of Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Texas 75235, and Nuclear Medicine Service, Veterans Administration Hospital, Dallas, Texas 75216

Abstract

Fatty acid synthesis from radiopropionate was evaluated in sural nerve biopsy slices from five normal controls and nine patients with pernicious anemia. The nerves were incubated in [14C]propionate, the lipids were extracted, and the fatty acid methyl esters were chromatographed by gas-liquid chromatography. In the normal nerves the radiolabel was found primarily in short chain (C12 and C14) fatty acids. The nerves from pernicious anemia patients showed two fatty acids peaks that were not discernible in the normal nerves, and these fatty acids had retention times intermediate to those of myristic (C14·0) and palmitic (C16·0) acids and palmitoleic (C16·1) and stearic (C18·0) acids, respectively. These two peaks (a C15 and C17 fatty acid) contained the bulk of the radioactivity recovered in the fatty acid fraction after incubation with [14C]propionate. Catalytic reduction and rechromatography failed to alter the retention time of these compounds suggesting that they are not unsaturated fatty acids. The nerves from the pernicious anemia patients had a decrease in the mean content of normal fatty acids when compared with the nerves from control patients as well as a decrease in the mean synthesis of normal fatty acids as estimated by isotope incorporation after incubation with [14C]propionate or [3H]water. Analysis of myelin isolated from the nerves indicated that the changes at least in part were in that fraction.

A portion of this material was presented at the 63rd Annual Meeting of the American Society for Clinical Investigation, Atlantic City, N. J., on 3 May 1971, and published in 1971 J. Clin. Invest. 50: 33a. (Abstr.)

Received for publication 24 June 1971 and in revised form 9 January 1972.

Introduction

The administration of vitamin B12 to patients with pernicious anemia (PA)1 results in correction of the hematopoietic abnormalities as well as stabilization or improvement in the neurologic lesions. It has been known for some time that folic acid administration is also capable of repairing the megaloblastic state (1) but that it fails to improve and may in fact worsen the neurologic disease (2, 3). The explanation for the folic acid effect is thought to be as follows: In man vitamin B12 is known to participate in the methyltransferase reaction (N4-methyltetrahydrofolate methyltransferase) where homocysteine conversion to methionine provides tetrahydrofolate for a variety of chemical reactions (Fig. 1) (4). The source of folate in this reaction is the storage form of folate, N4-methyltetrahydrofolate acid. The clinical expression of vitamin B12 deficiency, via interference with this pathway, is based upon the need of B12 as a coenzyme for the methyltransferase reaction. Thus B12 deficiency results in a "trapping" of this storage folate reducing available active folate coenzyme forms. Since active folate is required in the conversion of deoxyuridylate to thymidylate, deprivation interferes with the pyrimidine biosynthetic pathway of deoxyribonucleic acid producing the classic megaloblastic changes seen in B12 deficiency (5-7). As would be anticipated folic acid bypasses this alteration and effectively repairs the defect even in the continued absence of vitamin B12 (Fig. 1).

The second biochemical pathway that is affected by vitamin B12 deficiency is that responsible for the metabo-

1 Abbreviations used in this paper: GLC, gas-liquid chromatography. P.A., pernicious anemia.
Propionic acid → methylmalonyl CoA → Succinyl CoA

**Figure 1** The vitamin B₆-dependent biochemical reactions in man.

The biochemical basis of the neurologic lesion in pernicious anemia is not known. Since folic acid does not improve the neuropathy and since there are only two reactions known to require B₆ in man, it seems attractive to suppose that the neurological lesion in B₆ deficiency is associated with abnormal propionate metabolism. It is known that myelin lipids turnover at a significant rate (9–11) and that myelin renewal requires normal fatty acid synthesis (12). *De novo* fatty acid synthesis utilizes acetyl CoA as the anchor end of the growing fatty acid chain and malonyl CoA as the two-carbon donor during elongation. Substitution of propionyl CoA for acetyl CoA or methylmalonyl CoA for malonyl CoA would then lead to the formation of odd or branched chain fatty acids as suggested by Cardinale, Dreyfus, Auld, and Abeles (13). Conceivably, deranged fatty acid synthesis could result in abnormal myelin which in turn might be an accompaniment of the neuropathy of pernicious anemia.

For these reasons the present study was undertaken to evaluate propionate metabolism in the peripheral nerves of patients with pernicious anemia.

**METHODS**

**Clinical studies.** Biopsies of the sural nerve were performed prior to any therapy; informed consent was obtained. In one patient with pernicious anemia a biopsy was also obtained from the gluteal fat. A diagnosis of pernicious anemia was made in nine patients, ages 38–80 (of which two were beyond age 63), after a careful history and physical examination where there was evidence of a macrocytic anemia with hypersegmentation of the polymorphonuclear leukocytes. Bone marrow aspiration in all patients revealed classic megaloblastic erythroid hyperplasia with giant metamyelocytes. The serum vitamin B₁₂ levels (14, 15) were below 120 pg/ml (normal 200–800 pg/ml), and the serum folate levels (16) were greater than 4.0 ng/ml (normal 3.2–18 ng/ml). Gastric analysis revealed achylia gastrica with no change or a slight rise in the pH of gastric secretions following maximum histamine stimulation. Methylmalonic aciduria (17) was present in all the patients as was urinary excretion of propionic acid (18) in the five patients in whom the test was performed. Further documentation of the diagnosis was achieved by evidence of a reticulocyte response, repair of the anemia and reversion of the marrow to normoblastic maturation with "physiologic" doses of vitamin B₁₂ (1–10 μg/day i.m.). Subsequent
corroboration was made by Schilling tests (19) and intrinsic factor assays (20). Two nerves were obtained from patients with classical megaloblastic changes. Subsequent study demonstrated a normal serum B12 level and the absence of methylmalonic and propionic aciduria but reduced serum and red cell folate values and free gastric acid. The folate etiology was established by resultant complete hematologic repair with 50 µg of folic acid per day.

The control group of five patients, ages 46–62, was similarly studied. These patients were biopsied prior to amputations for malignant neoplasms of the thigh. On the basis of histological and clinical findings, there was no evidence of associated neuromuscular disease.

Preparation and characterization of the fatty acids. Immediately upon surgical removal of the segment of nerve a section was taken for light and electron microscopic study and determination of total lipids. The nerve was then placed directly into cold Krebs-Ringer bicarbonate buffer, pH 7.0, containing glucose at a concentration of 10 mM. The nerves were then transported to the laboratory, dissected free of surrounding connective tissue, checked under a dissecting microscope, weighed and serially sectioned at 0.5 mm intervals. Nerve slices were then incubated with shaking at 37°C in 3 ml of Krebs-Ringer bicarbonate buffer, pH 7.0. The buffer contained glucose (1 mg/ml) and 100 µmol of [14C]propionate (specific activity 35 mCi/mmole; Amersham/Searle Corp., Arlington Heights, Ill.), and the gas phase was 5% CO2-95% O2. Control flasks consisted of the above either without added tissue or with nerve that had been boiled for 30 min. In some experiments [14C]acetate (specific activity 61 mCi/mmole; Amersham/Searle) was utilized in place of propionate. Total fatty acid synthesis was estimated using tritiated water (21) in some experiments. Recovery of fatty acids in the isolation procedure was checked in control experiments by the addition of [14C]palmitate (New England Nuclear Corp., Boston, Mass.). Final results were corrected for this recovery, which was about 70% (66–76%)

After 2 h incubation the reaction was stopped by rinsing the tissue twice in cold buffer following which the flask contents were saponified by autoclaving with 2.0 ml of 90% KOH for 60 min (20 lb pressure, 250°C). Hexane extraction of the aqueous alkaline specimen was performed, and the upper (hexane) phase was separated and counted. The lower phase was then acidified with concentrated HCl to pH 1.0. After 10 min it was extracted three times with 30 ml of petroleum ether, evaporated to dryness, and made up to fixed volume. A portion was assayed by thin layer chromatography to document that only free fatty acids were present, and radioactivity was assayed (22, 23).

The sample was again evaporated to dryness, and methyl esters of fatty acids were prepared by the addition of 5 ml of anhydrous methanol and 0.1 ml of concentrated H2SO4, with refluxing for 30 min at 100°C on a sand bath. An equal volume of water was added, and the sample was extracted twice with petroleum ether. 2 ml of 0.1 M sodium bicarbonate was added to the extracts, and, following shaking, the petroleum ether extract was recovered and evaporated to dryness. A measured volume of acetonitrile was added, following which the material was assayed by gas-liquid chromatography (GLC).

Gas-liquid chromatography of the fatty acid methyl esters was performed on 6 ft columns using either a Barber-Coleman Model 10 chromatograph (Rockford, Ill.) or Model 5360 with a Packard Fraction Collector Model 852 (Packard Instrument Co., Inc., Downers Grove, Ill.). Diethylene glycol succinate (15%) was utilized as the stationary phase, and column temperatures were 165-170°C. Instrument standardization was performed by use of methyl esters of myristic, palmitic, and stearic acids (24) as well as with odd chain fatty acid esters (Applied Science Laboratory, State College, Pa.). The purity of the esters was checked by GLC. Individual peaks were collected for determination of radioactivity in a scintillator fluid consisting of 0.4 g p-his(2-(5-phenyloxyazolyl)]-benzene, 8 g 2,5-diphenyloxazole, 120 g naphthalene, 200 ml absolute methanol, and 40 ml of ethylene glycol made up to a liter with dioxane. Samples were counted in a Packard liquid scintillation counter, Model 314E. All specimens were counted to a standard error of 5% or less.

Where indicated the separated fatty acids were hydrogenated by the method of Kishimoto and Radin (25). In separate experiments completeness of the catalytic reduction process was documented.

Preparation and separation of myelin. Separation of myelin was carried out following the incubation of the nerve slices with radiolabeled propionate in some of the specimens. The preparation and separation of myelin was by the method of Smith (26). Because of the limited size of the nerve samples co-extraction with a large mass of peripheral nerve tissue obtained at autopsy was required. It was separately documented that the carrier nerve tissue was biologically inactive and would not incorporate either [14C]acetate or [14C]propionate into fatty acids. Myelin purity was evaluated by electron microscopy and residual protein content (10, 27).

RESULTS

Incorporation of [1-14C]propionate and [1-14C]acetate into normal nerve. The incorporation of 3C from labeled acetate and propionate into the saponifiable fraction of nerve was determined. Control (boiled) nerve synthesized no detectable fatty acids. In the intact nerve radiopropionate incorporation into fatty acids was only 20% that of radioacetate. Employing nerve slices [14C]acetate incorporation was 10–30% less than that of whole intact nerve, values similar to those reported by Majno and Karnovsky (28). However, the uptake of radiopropionate in the slices was approximately twice that of the respective intact nerves in two separate nerve studies.

The incorporation of radiopropionate was noted to depend upon the duration and conditions of storage in the interval between surgical excision and institution of the incubation, not unlike the circumstances affecting radioacetate incorporation (28). Nerve stored at room temperature in buffer for 3 h had approximately 30% of the incorporation of a similar portion incubated promptly upon excision. By contrast storage in cold buffer over the same interval resulted in incorporation of approximately 80% of the control values. The addition of glucose to the buffer during the storage period did not appear to enhance the incorporation significantly.

Isolation and characterization of the fatty acids from the nerves of normal and pernicious anemia patients. Gas chromatographic analyses of the methylated fatty acids were performed on nerves obtained from the four con-

Neural Fatty Acid Metabolism in Pernicious Anemia  1239
trol and seven patients with pernicious anemia. Nerves from one control and two patients with PA were utilized for the separation of myelin and are described below. The partition of fatty acids from the normal nerve is displayed in the upper panel of Fig. 3. The pattern obtained from the nerve in pernicious anemia patients was distinctly different (Fig. 3, bottom panel). At least two peaks were noted in the GLC analysis of fatty acids from the nerves from pernicious anemia patients that were not found in the normal nerves. One peak was evident between the myristic (C14·0) and palmitic (C16·0) and a second one just beyond palmitoleic (C16·1). The retention times for the normal fatty acids were same for the normal and for nerves obtained from pernicious anemia patients, with the exception of minor variation inherent in the method. In experiments not shown much larger quantities of fatty acids from control nerves were injected into the GLC. Even under these conditions or with increased sensitivity settings on the GLC, no peaks comparable to those seen in the nerves from pernicious anemia patients could be identified. In addition, these peaks were not demonstrated in the two nerves obtained from the patients with folate deprivation.

Tentative identification of the two fatty acids that primarily incorporated the radiopropionate (see Fig. 3) was obtained by comparison with the GLC retention times of known methyl fatty acid standards. The unknown peak identified between myristic (14·0) and palmitic (16·0) acids had a retention time and pattern seen with anti-iso-C15 standards; that noted between palmitoleic (16·1) and stearic (18·0) acids was compatible with that of a C17 fatty acid.

Another means of characterization of these fatty acids is shown in Fig. 4. Following chromatographic analysis

**Figure 3** Gas-liquid chromatographic isolation of fatty acids. The upper panel represents the GLC pattern of a normal nerve. The radioactivity is denoted by the (---) lines and hatched pattern. The lower panel is the pattern obtained from a nerve from a patient with pernicious anemia. Identical conditions of temperature and gas flow were present in both studies. A very slight difference in retention times was seen in this nerve from a patient with PA as compared with the control nerve. In general, however, the chromatograms of the PA patients were superimposable upon those of the control patients. The predominant radioactivity was noted between the C14 and C16 fatty acids and between the C16·1 and C18 fatty acids.

**Figure 4** Effect of addition of standards. The upper panel depicts the GLC of a portion of nerve from a pernicious anemia patient. The middle panel is the C15 standard. The lower panel is the repeat chromatography of another portion of the nerve to which the C15 standard was added. Identical GLC conditions were maintained.
TABLE I

**Mean Content** of Fatty Acids in Nerves From Normal and Pernicious Anemia Patients

<table>
<thead>
<tr>
<th></th>
<th>Dry weight</th>
<th>Total lipids</th>
<th>Fatty acids</th>
<th>C14 through C18 fractions of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>mg</td>
<td>µg</td>
<td>µg</td>
</tr>
<tr>
<td>Normal (4)†</td>
<td>42</td>
<td>25.8</td>
<td>(61.5%)†</td>
<td>573 Not detectable</td>
</tr>
<tr>
<td>Pernicious anemia (7)</td>
<td>36.5</td>
<td>11.1</td>
<td>(30.4%)</td>
<td>(±135)</td>
</tr>
<tr>
<td>Folic acid deficiency (2)</td>
<td>45</td>
<td>27</td>
<td>(60.2%)</td>
<td>(±131)</td>
</tr>
</tbody>
</table>

* Content per 100 mg wet weight.
† Number of patients studied.
§ % of dry weight as lipids.
∥ SEM.
† Mean values.

of a portion of fatty acids from a nerve obtained from a pernicious anemia patient, an anti-iso-C15 standard (noted in the middle panel of Fig. 4) was added to a second portion of the sample. Chromatography of that portion is recorded in the lower panel of Fig. 4, which depicts a reasonable addition of the anti-iso-C15 standard to the unknown peak and symmetry of that peak suggesting homogeneity and identity of these fatty acids.

Further characterization of these two fatty acids was performed by hydrogenation. Since catalytic reduction is known to reduce unsaturated fatty acids, hydrogenation of the isolated fatty acid methyl esters was done. As displayed in Fig. 5 the isolated fatty acids had the same retention times when rechromatographed following hydrogenation as they did prior to hydrogenation, a finding that supports the suggestion that the original fatty acids were saturated.

Finally, preliminary mass spectroscopy of these two peaks is consistent with their being a C15 and a C17 fatty acid. Further characterization of these fatty acids is currently in progress.

**Quantification of the fatty acids.** Total lipids determined in portions of nerves from normal patients was 54.6-66% (mean 61.5%) and from patients with pernicious anemia 29.8-52% (mean 30.4%) expressed as a percentage of the dry weight. Electron microscopic abnormalities were greatest in those nerves with lowest total fat content.

The net contents of the individual methylated fatty acids in the normal, folate deprived, and pernicious anemia nerves are compared in Table I. Of the total fatty acids, 76% in the control, and 72% in the FA nerves were in the C14 through C18 fractions; in both cases about 20% were C20 or longer. Considerable variation was seen in the normal nerves as is reflected in the standard error of the mean in two representative normal fatty acids (C14-0, myristic acid; C16-0, palmitic acid) that had GLC retention times near the odd numbered fatty acids. In spite of this variation in the normal, the nerves from the pernicious anemia patients had a statistically significant decrease in content of these normal fatty acids.

![Figure 5](https://example.com/figure5.png)

**Figure 5** Catalytic reduction of the separated “abnormal” methylated esters of fatty acids. On the left is a GLC separation of a nerve from a pernicious anemia patient. The peaks A and B were collected and subjected to hydrogenation. Rechromatography following hydrogenation is shown on the right. The GLC retention time of these two peaks is essentially the same as that noted in the whole nerve.

---

1. Mass spectroscopy was performed on a Varian Mat GmbH GC/MC system Mat III "Gnom" by R. Carr of Varian Associates, Palo Alto, Calif.
fatty acids when analyzed by the Mann-Whitney U test (29). The abnormal fatty acids (C15 and C17) were identified only in the pernicious anemia nerves.

**Synthesis of fatty acids from nerves of normal and pernicious anemia patients.** The incorporation of [14C]propionate into the isolated fatty acids in the nerve from the normal patients is shown in the upper panel of Fig. 3. Radioactivity was identified primarily in myristic (C14-0) acid, although a small number of counts were also noted in palmitic (C16-0), lauric (C12-0), and capric (C10-0) acids. The pattern of radioactivity in the isolated fatty acids from the nerve of a pernicious anemia patient is shown in the lower panel of Fig. 3. The two abnormal fatty acid fractions contained most of the radioactivity identified in these nerves. Significant radioactivity was not identified beyond C18-0, except for one nerve from a pernicious anemia patient which contained a small number of counts in a fatty acid with a very long GLC retention time, well beyond arachidonic acid (C20-4). Characterization of this fatty acid peak is now in progress.

The mean synthesis of fatty acids from [14C]propionate is recorded in Table II. A significant decrease in the synthesis of the normal (C14 and C16) fatty acids with propionate as the precursor was seen in the pernicious anemia nerves. Again, the incorporation of the radiopropionate into the C15 and C17 fatty acids was seen only in the pernicious anemia nerves.

In experiments not shown determination of fatty acid synthesis by the 3H20 method in two normal nerves was 1322 and 1120 nmol of 3H-equivalents (21) incorporated per 100 mg of nerve and 620 and 920 nmol for two nerves obtained from pernicious anemia patients.

**Studies of separated myelin.** Electron microscopic examination of the separated myelin preparations demonstrated the classical disrupted lamellar membrane pattern of myelin. Other recognizable structures were absent. Studies performed in one normal and two nerves from B12 deficient patients revealed 22% of the original counts identifiable in the separated myelin of the normal and 31 and 37% in that of the PA nerves. All of the radioactivity was in C16-0 (palmitic acid) in the myelin from the normal nerve and it was found in C15 as well as C16-0 fatty acids in the myelin from the PA nerves.

Incubation of adipose tissue obtained from the gluteal region of one patient with pernicious anemia with radiopropionate demonstrated only a small incorporation of the labeled precursor into extractable lipids and GLC did not demonstrate the C15 or C17 fatty acids.

Thus, in the presence of vitamin B12 deficiency the net content of normal fatty acids was reduced, and the synthesis of normal fatty acids with propionate as the precursor was decreased when compared to normal nerves. In addition, the presence and synthesis of the abnormal fatty acids (C15 and C17) were identified only in the pernicious anemia nerves.

**DISCUSSION**

The present study evaluated certain aspects of fatty acid metabolism in nerve slices obtained from sural nerve biopsies from five normal subjects and nine patients with classical pernicious anemia utilizing propionate as the substrate (30-32). As a result of these studies four general conclusions can be drawn. First, as noted in Table I, the nerves from patients with pernicious anemia had a decrease in content of normal fatty acids. Second, although the synthesis of normal fatty acids (e.g. C14-0 and C16-0) from propionate was demonstrable in nerves from B12-deficient subjects, total fatty acids synthesis was decreased when compared with the normal nerves determined by both the tritiated water method and with radiopropionate as the precursor. This decrease in net synthesis was statistically significant in spite of considerable variation from nerve to nerve. Such variation would be anticipated from the previous evidence (28) that lipogenesis is not completely uniform even in mature nerves. The fact that fatty acid synthesis was decreased suggests that the diminished content of fatty acids in the nerves was not the simple consequence of
myelin loss. Third, the nerves from the pernicious anemia patients demonstrated two apparently abnormal fatty acids. One was tentatively identified as a C15 and the other as a C17 fatty acid. Finally, the present studies demonstrated that the nerve in pernicious anemia is capable of incorporating [14C]propionate into these putative odd chain fatty acids during in vitro incubation.

It cannot be said unequivocally on the basis of the studies reported here that the two new fatty acid peaks occurring in the gas-liquid chromatograms of nerves from patients with pernicious anemia are "abnormal" or give rise to the neural lesion seen in this disease. Fatty acids with apparently similar retention times have been reported in rat adipose tissue extracts by Horning, Martin, Karmen, and Vagelos (32), and trace amounts at C15 and/or C17 fatty acids have been found in nerves from several species of animals (33, 34). On the other hand the possibility also exists that they are unique. This follows from the observation that isotope from propionic acid was recovered in the two peaks after incubation with nerves from patients with pernicious anemia but not from normal controls. Thus if equivalent fatty acids were present in normal nerves the amount would have to be vanishingly small to be below the limits of detection by either isotopic or GLC assays. At the very least it can be said that quantitative differences in their concentrations are large. Further support for specificity is based upon the absence of similar peaks in the megaloblastic anemia patients with folic acid deficiency. In addition, in experiments not shown one nerve from a patient with a hypoproliferative anemia secondary to chronic renal failure also did not demonstrate the changes seen in the PA patients.

In this regard it is of interest that similarly abnormal fatty acids have been found in Corynebacterium simplex grown in B12-deficient media (35). Similarly glial cells grown in tissue culture in a B12-deprived state have recently been reported to produce C15 and C17 fatty acids, and these fatty acids disappeared when B12 was made available (36). However, since glial cells have a scavenger function and since the primary cellular site of abnormality in the neurologic lesion of vitamin B12 deficiency in man is the oligodendrocyte (37), the relationship to the present studies may not be direct. Finally, a recent case of congenital propionic acidemia was reported in a neonate who died at 8 days of age with prominent central nervous system damage (38). An abstract has suggested the defect was at the level of propionyl carboxylase and studies of the liver have demonstrated an accumulation of C17 fatty acids (39).

It should be noted that the present fatty acid studies were carried out on nerve slices which contain a variety of cell types. Since the neurological lesion in pernicious anemia appears to be one of dysmyelinization (37, 40), it was important to know that myelin fatty acids were abnormal. The small quantity of tissue available made this difficult. Nevertheless in the control and two PA nerves where it was possible to isolate myelin after incubation with radiopropionate, the patterns were similar to that seen in whole nerve.

Although the basis for the neurologic lesion in pernicious anemia is unknown, the present observations provide a possible explanation for this defect. In animals myelin has been demonstrated to be in a dynamic state of turnover, and certain lipids (inositol phosphatide and lecithin) are known to turn over more rapidly than others (9, 10). It has been suggested that the formation and integrity of myelin is dependent upon the quantity of specific fatty acids available (12, 28). The present study has shown that in the presence of a metabolic intermediate (propionic acid) which is known to accumulate in pernicious anemia, there is a decrease in net synthesis of normal fatty acids in neural tissue. In addition, unusual fatty acids appeared to be synthesized. Thus, decreased synthesis of normal fatty acids and the presence of abnormal fatty acids could result in altered myelin integrity and renewal, or dysmyelinization, which might in time result in defects in neuron function.

It must be emphasized that this formulation, while attractive, is entirely tentative. One source of concern is the observation that not all children with methylmalonic aciduria, a congenital syndrome with a defect in the conversion of methylmalonyl CoA to succinyl CoA (41-46) equivalent to the B12 deficiency lesion, have neurological disease of the type seen in fully developed pernicious anemia. On the other hand a number of the patients have had mental retardation and peripheral neuropathy (44). It is conceivable that failure to develop neuropathy indicates a milder or incomplete defect just as the adult with acquired B12 deficiency may have a variable expression of that state. Under any circumstance, the changes in fatty acid metabolism in the peripheral nerves of patients with pernicious anemia are of interest in themselves and hopefully will lead to a better understanding of the pathophysiology involved.

ACKNOWLEDGMENTS

I wish to express gratitude to Mrs. Frances Woelfin and Dorothy Lu for excellent technical assistance. This study was supported in part by the Adam Foundation.
REFERENCES


E. P. Frenkel