Familial Hyperglycerolemia

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ABSTRACT A 70-yr-old mildly diabetic white male was discovered to have an elevated level of serum free glycerol in the range of 75 mg/dl and to excrete about 13 g of free glycerol in the urine per 24 h. During a 24-h fast the urine glycerol loss increased to 21.5 g per 24 h. Studies carried out in vitro using leukocytes prepared from the patient's blood which were incubated with [^{14}C]glycerol demonstrated an almost complete absence of glycerol oxidation to $^{14}CO_2$ and of glycerol phosphorylation, in contrast to control studies with leukocytes collected from normal subjects. Homogenates of the patient's leukocytes contained negligible activity of ATP:glycerol phosphotransferase (glycerokinase EC 2.7.1.30) as measured by a direct spectrophotometric method. Marked hyperglycerolemia has thus far been detected in one brother and in one son of the daughter of this patient. This evidence suggests an x-linked recessive inheritance pattern of the trait. There is a high prevalence of diabetes mellitus in this family.

INTRODUCTION

Free glycerol[1] is normally present in plasma at levels below 1.5 mg/dl. Its endogenous source is lipolysis of triglyceride in adipose tissue, and it is removed from the blood largely by the liver and kidneys (1–4). After phosphorylation, it may enter the glycolysis pathway or be utilized for synthesis of glycerolipid. The plasma glycerol level rises only slightly in fasting subjects in spite of marked increases in its rate of production (5), indicating that the mechanism for its removal normally has a high reserve capacity.

We have recently observed an adult male who demonstrates a markedly increased plasma free glycerol level in the range of 75 mg/dl, and a daily loss of about 13 g of free glycerol in the urine.

This paper contains a description of the major biochemical features of this patient as well as the results of experiments in which the metabolism of [U-^{14}C]glycerol was studied in leukocytes isolated from his blood. The findings indicate that there is a failure of glycerol utilisation due to absence of ATP:glycerol phosphotransferase (glycerokinase) activity.

METHODS

Case report

The patient, a 70-yr-old white male, has been known to have mild diabetes mellitus since 1967 and has suffered myocardial infarctions in 1968 and again in 1971. His medical history also includes a laryngeal carcinoma *in situ* treated by cobalt irradiation in 1971 and without evidence of recurrence or metastasis. Osteoarthritis with particular involvement of the knees has been present for several years. In 1970, he had an attack of herpes zoster ophthalmicus which resolved completely within 2 wk. There is a strong family history of diabetes mellitus (his mother and 4 of 11 siblings).

At the time of the initial laboratory study which led to the present investigation, he was receiving the following medications: sulfinpyrazone 50 mg q.i.d., clofibrate 500 mg b.i.d., phenylbutazone 100 mg daily, digoxin 0.25 mg daily. A blood specimen was submitted to a laboratory for routine measurement of total cholesterol, triglyceride, and lipoprotein electrophoresis.[2] The serum triglyceride was measured enzymatically (6) by the Stat-Pak method (Calbiochem, San Diego, Calif.) in a centrifugal analyser (Centrifichem, Union Carbide Corp., Rye, N.Y.). This procedure was initially performed without the inclusion of an unhydrolyzed serum blank, which is standard practice because of the normally negligible contribution to the triglyceride result from the low level of serum free glycerol. A very high apparent serum triglyceride value of 966 mg/dl was obtained. This did not coincide with the lipoprotein electrophoresis pattern which indicated only a moderate type IV hyperlipidemia with absent chylomicrons. In addition the serum did not appear lipemic. This discrepancy prompted further investigation including measurement of the free glycerol level on unlipolysed serum as well as measurement of the serum triglyceride using a chemical extraction method which does not detect free glycerol (7). Serum total cholesterol was measured by a standard semi-automated chemical procedure (8).

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[1] The term free glycerol is used here to denote glycerol not associated with any chemical group, including phosphate.

[2] The initial laboratory studies on this patient were performed at the London Biochemistry Reference Laboratory Inc., London, Ontario.

Further studies were carried out with the patient in hospital, where he remained ambulatory and consumed a regular diet. Upon admission, all medications were withdrawn except for digoxin. Physical examination revealed a 5'6" male weighing 145 lbs and in apparently good health. The blood pressure was 130/74 mm Hg and apart from bruits over the femoral arteries, no specific abnormal physical findings or organomegaly was present.

An SMA 12/60 chemistry profile (glucose, total protein, albumin, calcium, phosphorus, alkaline phosphatase, uric acid, serum glutamic oxaloacetic transaminase, lactic dehydrogenase (LDH), creatinine, blood urea nitrogen, and total bilirubin) carried out on fasting serum was within normal limits. Endocrine assessment included measurement of the following serum parameters by standard radioassay procedures in the fasting state: thyroxine, resin-T3 uptake, thyroid-stimulating hormone, growth hormone, and cortisol (A.M. and P.M.), and these were all normal. A 100 g, 5-h oral glucose tolerance test was carried out after a 14-h fast, and in addition to glucose (9), the following measurements were made on blood taken at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 h: glycerol, non-esterified fatty acids (NEFA) (10), β-hydroxybutyrate (11), lactate (12), glucagon, using Unger antibody 30K (13), and insulin (14). Serum electrolytes and bicarbonate were determined using the Stat-Ion procedure (Technicon Instruments Corp., Tarrytown, N.Y.). Serum osmolality was measured by freezing-point depression (Precision Systems, Inc., Sudbury, Mass.).

Identification of free glycerol in serum and urine

Serum glycerol.[3] The Calbiochem triglycerides-glycerol method which was initially employed to measure the serum triglyceride (TG) and glycerol is based on phosphorylation of free glycerol by glycerokinase (GK) as shown below:

$$\text{TG} \xrightarrow{\text{L}} \text{glycerol} + \text{NEFA}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol-1-P} + \text{ADP}$$

$$\text{Phosphoenolpyruvate} + \text{ADP} \xrightarrow{\text{PK}} \text{pyruvate} + \text{ATP}$$

$$\text{Pyruvate} + \text{NADH}^+ + \text{H}^+ \xrightarrow{\text{LDH}} \text{lactate} + \text{NAD}$$

(L = lipase). Because glycerokinase can also phosphorylate glyceraldehyde and dihydroxyacetone (15), the possibility that instead of glycerol, one of these other reacting substances was present in the blood could not be excluded by the glycerokinase method alone. Therefore an alternative serum glycerol assay employing the highly specific enzyme glycerophosphate dehydrogenase (GPDH) (EC 1.1.1.8) (Boehringer Mannheim Corp., Montreal, Canada) was carried out:

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol-1-P} + \text{ADP}$$

$$\text{Glycerol-1-P} + \text{NAD}^+ \xrightarrow{\text{GPDH}} \text{dihydroxyacetone-P} + \text{NADH} + \text{H}^+$$

[3] The initial measurements were performed on serum and the later ones on plasma; no significant difference in glycerol content of serum vs. plasma was observed.

Urine glycerol. The patient's urine was found to contain about 13 g per 24 h of the material suspected to be free glycerol. The urine was subjected to thin layer chromatography on microcrystalline cellulose (Sigma Chemical Co., St. Louis, Mo.) with [U-^{14}C]glycerol (46 mC/mM) and [U-^{14}C]glycerophosphate (21.5 mC/mM) obtained from Amersham/Searle Corp., Arlington Heights, Ill., added as markers. The plates were developed in isobutanol:ammonia:water (60:30:10 vol/vol) and were sprayed with alkaline permanganate to detect glycerol (16) and with sulfosalicylic acid-ferric chloride to detect phosphates (17). In preliminary studies, the latter method was found sufficiently sensitive to detect 0.025 mg of glycerol-1-phosphate per liter of urine.

The abnormal component was separated from other urine constituents by application of 3 ml of undiluted urine (containing [^{14}C]glycerol as a marker) onto a G10 Sephadex column (90 × 1.5 cm) (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) followed by elution with distilled water at a rate of 14 ml per h. The radioactivity was recovered in a single peak which emerged at 6.4 h, and enzymatic assay showed that this fraction contained over 90% of the urine component suspected to be free glycerol. Chemical assay demonstrated it to be virtually free of glucose, urea, and creatinine. The syrupy residue remaining after concentration of the fraction under reduced pressure was analyzed at 99.9 mHz in a Varian MA-100 nuclear magnetic resonance spectrometer (Varian Associates, Instruments Div., Palo Alto, Calif.). For comparison, pure glycerol (Fisher Scientific Co., Pittsburgh, Pa.) was analysed in the same manner.

Because of the well known effect of fasting as a stimulant to increased glycerol production, a further experiment was carried out to observe the effect of fasting upon this patient's plasma and urine glycerol levels. For a 3-day period, blood specimens were taken at 8 A.M. and 8 P.M. for measurement of glycerol, glucose, and creatinine. The morning specimens were taken before breakfast on days 1 and 3. Urine was collected in 12-hour aliquots from 8 A.M. to 8 P.M. and from 8 P.M. to 8 A.M. Regular food intake was permitted until 8 P.M. on day 1 when fasting began. Fasting with unrestricted water intake was continued to 8 A.M. on day 3 when normal food intake was resumed.

Glycerol metabolism in isolated leukocytes

Whole blood taken from the patient and from non-hyperglycerolemic controls was collected into heparinized syringes. The controls were males who were free from apparent infectious or metabolic disease. Their ages ranged from 22 to 74 yr. The leukocytes were immediately isolated by dextran sedimentation (18) and were washed thoroughly by three repeated centrifugations and resuspensions of the leukocyte pellet in 5-ml vol of Tris-buffered Hank's solution, pH 7.4 before final suspension in 4 ml of this medium. Aliquots were taken for cell counting (Coulter model S, Coulter Electronics, Inc., Hialeah, Fla.) before incubation.

The leukocyte suspensions were incubated in standard Warburg flasks at 37°C in the presence of (*a*) 1 μCi of [U-^{14}C]-glycerol without added carrier at a glycerol concentration of 5.4 μM, (*b*) 5 μCi of [U-^{14}C]glycerol plus added glycerol at a final concentration of 108 μM, and (*c*) 5 μCi of [U-^{14}C]glucose at a glucose concentration of 2.8 mM. After 120 min, 0.5 ml of 3 N H_2SO_4 was added from the sidearm, and CO_2 was trapped over a 16-h period in 30% KOH contained in the centre well. The radioactivity in the KOH was counted by liquid scintillation in Bray's solution (19) using a Packard model 3302 liquid scintillation spectrometer (Packard Instrument

Co., Inc., Downers Grove, Ill.). Quench correction was performed by the channels ratio method.

To study the ability of the isolated leukocytes to phosphorylate glycerol, leukocyte preparations from normal subjects and from the hyperglycerolemic patient were prepared as described and incubated for 120 min in the presence of 1 μCi of [U-^{14}C]glycerol. 4 vol of hot (70°C) absolute ethanol followed by 15 vol of chloroform were then added and mixed thoroughly. After standing overnight the clear aqueous phases were applied to columns of Dowex-1 (formate form, 0.9 × 7.0 cm, Dow Chemical Co., Midland, Mich.) which had been thoroughly washed with distilled water. They were then eluted with 6 ml of distilled water followed by 6 ml of 6 N formic acid. 1-ml fractions were collected and counted. Preliminary studies using mixtures of [U-^{14}C]glycerol and [U-^{14}C]glycerophosphate showed over 90% recovery of the former in the aqueous eluate while the latter was quantitatively recovered in the formic acid eluate.

Direct spectrophotometric measurement of glycerokinase activity in leukocytes taken from the hyperglycerolemic subject and from 10 normal subjects was performed after homogenization of the isolated leukocytes in 0.01 M acetate buffer, pH 5.4 with a Potter-Elvehjem homogenizer (20). Total protein in the homogenate was measured by the biuret technique (21).

RESULTS

The lack of agreement between the initial enzymatically determined, uncorrected serum triglyceride result of 966 mg/dl, the absence of serum lactescence, and the relatively minor increase in the serum prebeta lipoprotein suggested that the initial serum triglyceride result was erroneously high. As shown by the results given in Table I, this high value was found to be due to the presence of a substance in the serum which reacted when the enzymatic triglyceride assay was performed without prior lipolysis of the serum triglycerides. When the result was corrected for this high blank value, a true serum triglyceride level of 323 mg/dl was obtained. This is comparable to the value of 308 mg/dl given by the solvent extraction method that is free from interference by water soluble constituents of serum.

Although it was considered highly probable that the identity of the enzymatically reactive substance was free glycerol, this could not be stated definitively on the basis of its reactivity in the Calbiochem triglycerides-glycerol assay, because this procedure utilises the nonspecific enzyme, glycerokinase. The finding of a similar value for free glycerol when the highly specific assay system utilizing glycerophosphate dehydrogenase was used (Table I) provides confirmatory evidence for the identity of the reacting material being free glycerol.

Preformed α-glycerophosphate does not contribute to the glycerol result when the glycerokinase procedure is used, but it is included when the free glycerol is measured by the glycerophosphate dehydrogenase technique. The fact that similar results were obtained by these two techniques (Table I) indicates therefore that the plasma glycerol of the patient was entirely in the non-phosphorylated form.

Positive identification of glycerol was provided by the studies carried out on the urine of the patient. Thin layer chromatography of the patient's urine demonstrated that the component behaved identically to free glycerol in terms of its R_f value and staining characteristics and that glycerophosphate was not detectable. Finally, purification of the urine component over Sephadex G10 followed by nuclear magnetic resonance analysis demonstrated conclusively its identity to be free glycerol (Fig. 1).

The glucose tolerance test (Table II) indicates the presence of mild diabetes mellitus as judged by the prolonged elevation of the blood glucose, the subnormal value at 5 h, and the delayed prolonged serum insulin response. It can also be seen from Table II that both the serum non-esterified fatty acid and the β-hydroxybutyrate levels fell in response to the glucose administration while the plasma glycerol level remained uniformly high. There was a sustained rise in blood lactate. Apart from a brief rise at 0.5 h, there was no change in the plasma glucagon level which was in fact consistently somewhat below the expected range of normal for this method (100–135 pg/ml).

As shown in Table III, leukocytes from the patient produced negligible amounts of $^{14}CO_2$ when incubated with [U-^{14}C]glycerol whereas leukocytes from normal subjects were found to consistently produce $^{14}CO_2$

TABLE I
Laboratory Findings in Hyperglycerolemia

	Result	Normal range
Serum, *mg/dl*		
Total cholesterol	208	150–260
Triglycerides*	966	30–160
Triglycerides‡	323	30–160
Triglycerides§	308	50–150
Free glycerol‖	70	<0.5
Free glycerol¶	74	<0.5
Electrolytes, *meq/liter*		
Sodium	137	133–148
Potassium	4.7	3.5–5.5
Chloride	99	95–106
Bicarbonate	29	24–35
Osmolality, *mosmol/liter*	307	285–295
Urine		
Free glycerol,** *g/24 h*	13.0, 13.4, 13.3	Absent

* Uncorrected for serum blank.
‡ Corrected for serum blank.
§ Nonenzymatic colorimetric method.
‖ Glycerokinase enzymatic method.
¶ Glycerophosphate dehydrogenase enzymatic method.
** Determined on 3 successive days, patient non-fasting.

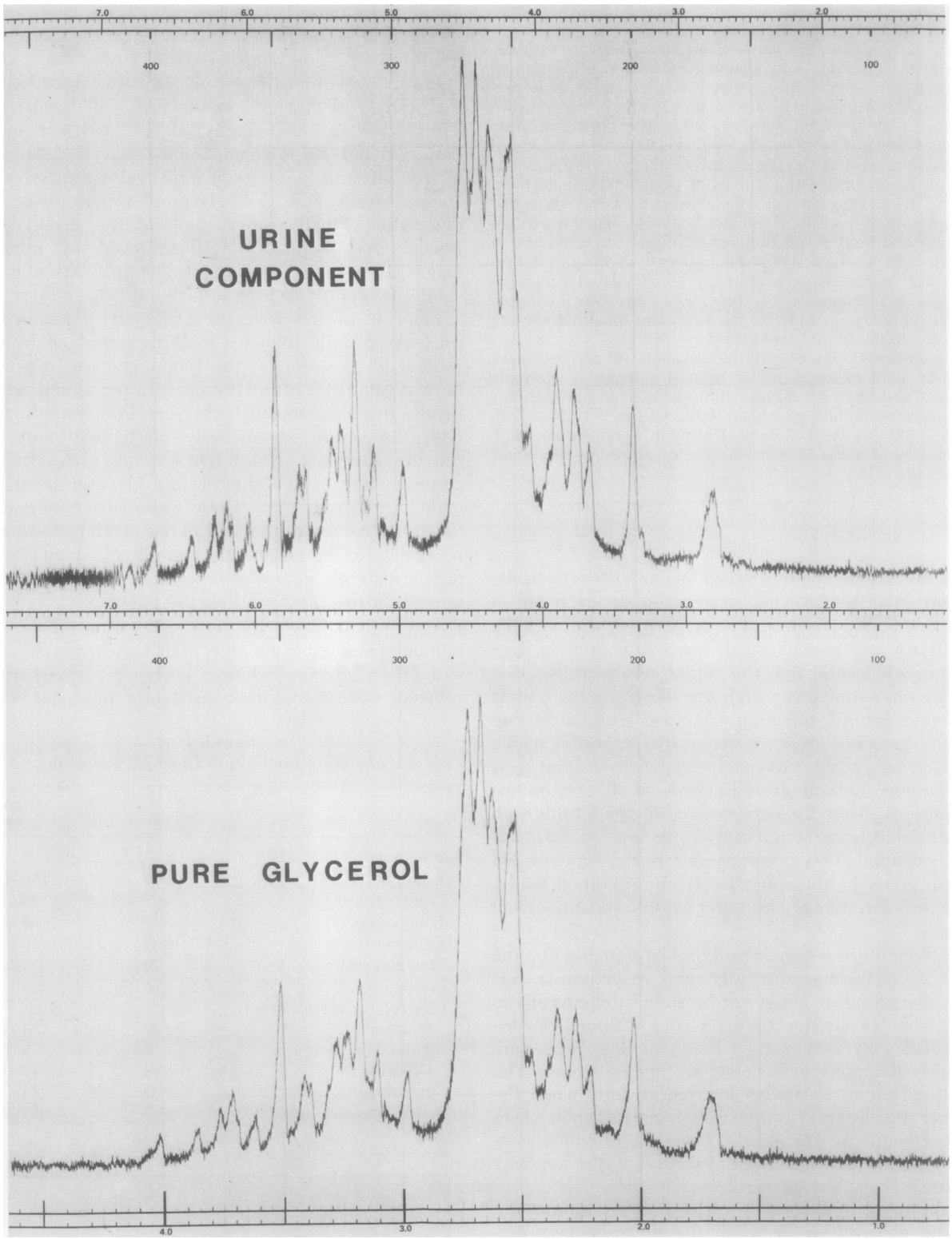

FIGURE 1 Nuclear magnetic resonance scan of the material isolated from the urine of the patient by chromatography over G10 Sephadex. The scan was performed in a Varian MA-100 nuclear magnetic resonance spectrometer at 99.9 mHz. The lower scan was carried out using pure glycerol. The identical appearance of the two scans proves that the urine component is glycerol.

TABLE II

Changes in Serum Constituents after a 100-g Oral Glucose Load

Time	Glucose	Insulin	NEFA	Glycerol	Lactate	Glucagon	β-Hydroxy-butyrate
h	*mg/dl*	*μU/ml*	*mM*	*mg/dl*	*mM*	*pg/ml*	*nM*
0	85	11	0.82	68	0.96	45	0.90
0.5	185	73	0.78	70	1.35	96	0.41
1.0	160	115	0.53	64	1.60	62	0.35
1.5	175	100	0.55	70	1.50	69	0.05
2	176	130	0.67	69	1.18	58	0.10
3	155	155	0.44	70	1.63	59	0.05
4	60	43	0.46	63	1.12	65	0.06
5	43	11	0.79	68	0.83	63	0.04

when incubated under identical conditions. When leukocytes from normal subjects were incubated for 120 min with [U-^{14}C]glycerol, significant amounts of the radioactivity were recovered in the glycerophosphate fraction. In contrast, the patient's leukocytes failed to convert the tracer into a phosphorylated product (Table III). The fact that the patient's leukocytes produced a similar amount of $^{14}CO_2$ as did the leukocytes of the control subjects when incubated with [U-^{14}C]glucose is indication that no defect in their ability to utilise glucose was present and that they retained metabolic activity under the conditions of handling and incubation.

The results of the measurement of urine glycerol during fasting for 24 h as shown in Table IV indicate that during the fasting period, the 24-h urine glycerol output increased from 12.0 to 21.5 g and that it fell to 12.5 g after normal food intake was resumed. The plasma free glycerol level did not increase during the fast, but for reasons not yet known, it was higher on the subsequent day. At the conclusion of the fasting period, the plasma glucose level was 80 mg/dl.

A survey of surviving family members has shown hyperglycerolemia to be present in one brother and in one grandson of the propositus. The family pedigree is shown in Fig. 2.

DISCUSSION

This patient exhibits a consistent and pronounced elevation in the plasma level of free glycerol and, in the non-fasting state, a daily urine glycerol loss of about 13 g. Apart from mild diabetes, the laboratory findings fail to reveal any evidence of an endocrine or metabolic disturbance which could cause glycerol over-production. Instead all of the findings in this patient point to failure of the tissues to metabolise glycerol which is being produced in apparently normal amounts.

When the patient's leukocytes were incubated in the presence of [U-^{14}C]glycerol at glycerol concentrations of 5.4 and 108 μM, it was found consistently that they failed to produce significant amounts of $^{14}CO_2$, whereas similarly treated leukocytes from normal subjects

TABLE III

In Vitro Studies with Leukocytes from the Hyperglycerolemic Patient and from Normal Controls

	Radioactivity in CO_2*			Radioactivity in glycerophosphate fraction	Glycerokinase activity in WBC
	(a)	(b)	(c)		
	dpm/10⁶ WBC			*dpm/10⁶ WBC*	*mU/mg protein*
Patient	80	76	15,200	37	<0.1
Normals	1,070 to 6,460‡	2,856 to 21,420§	11,200 to 29,300‖	4,447 to 7,387¶	0.46 to 1.22**

* Leukocytes (WBC) were incubated in 4 ml of Tris-buffered Hank's solution, pH 7.4 at 37°C for 120 min in the presence of (a) 1.0 μCi of [U-^{14}C]glycerol at a glycerol concentration of 5.4 μM/liter, (b) 5.0 μCi of [U-^{14}C]glycerol at a glycerol concentration of 108 μM/liter, and (c) 5.0 μCi of [U-^{14}C]glucose at a glucose concentration of 2.8 mM/liter.

‡ Range for nine non-hyperglycerolemic subjects.

§ Range for 11 non-hyperglycerolemic subjects.

‖ Range for five non-hyperglycerolemic subjects.

¶ Range for four non-hyperglycerolemic subjects.

** Range for 10 leukocyte preparations determined spectrophotometrically as described in the text.

TABLE IV
Effect of 24-h Fasting on Serum and Urine Glycerol Levels in the Hyperglycerolemic Patient

		Urine glycerol	Plasma glycerol*	Plasma glucose*	Creatinine clearance
		g	*mg/dl*	*mg/dl*	*ml/min*
	8 A.M.	} 5.4	72.8	126	59
Day 1	8 P.M.	} 6.6 (12.0)‡	81.1	140	
	8 A.M.	} 11.4	71.6	110	
Day 2§	8 P.M.	} 10.1 (21.5)‡	67.7	82	57
	8 A.M.	} 5.7	82.7	80	
Day 3	8 P.M.	} 6.8 (12.5)‡	102.6	150	53
	8 A.M.	}	89.8	120	

* 8 A.M. samples were taken before breakfast on days 1 and 3.
‡ Figures in parentheses indicate the total 24-h glycerol excretion.
§ Water only was consumed throughout day 2.

invariably resulted in $^{14}CO_2$ production. It was also observed that, in contrast to the controls, the patient's leukocytes failed to convert [U-^{14}C]glycerol into a phosphorylated product. On the other hand, there was no defect apparent in the ability of the leukocytes of the patient to produce $^{14}CO_2$ from [U-^{14}C]glucose which indicates that the observed metabolic defect is specific for glycerol. These findings are explicable on the basis of deficient glycerokinase activity in the patient's leukocytes. This was confirmed by direct spectrophotometric assay of the activity of this enzyme in leukocyte homogenates. Leukocytes from normal subjects consistently demonstrated glycerokinase activity, whereas only negligible activity was found in leukocytes from the patient (Table III).

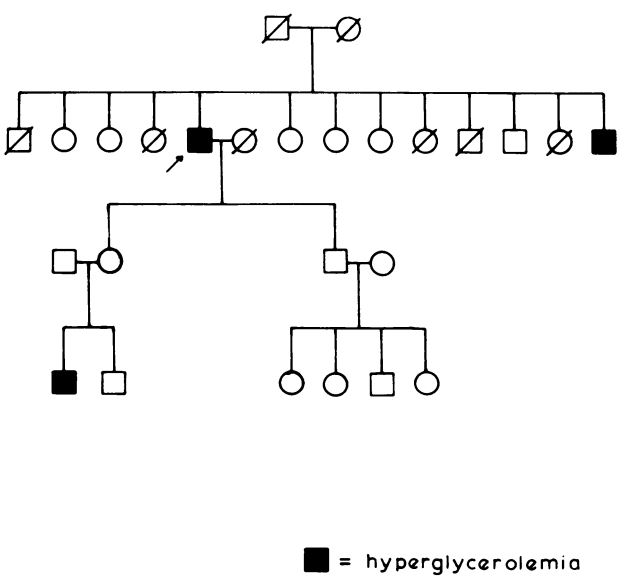

FIGURE 2 Pedigree of the hyperglycerolemic family. Males are indicated as squares and females as circles. The propositus is designated by the arrow. Oblique lines indicate relatives who were deceased before this study began.

Evidence for a generalized failure of glycerol metabolism in this patient was provided by the measurements of the urine glycerol output. In the fed state, the daily urine glycerol loss amounted to about 13 g. This increased to 21 g during fasting for 24 h and returned promptly to the initial level upon restoration of food intake (Table IV). The total glycerol production rate in lean fasting subjects has been measured by Bortz et al. by means of an isotope dilution technique (22). They found the glycerol turnover to be between 71.4 and 149.4 μmol per minute after a brief (6–13 h) interval of fasting which corresponds to production rates for free glycerol ranging from 9.5 to 19.8 g per 24 h. Owen et al. (23) estimated the total free glycerol utilization in liver and kidney by measuring the arteriovenous difference in blood glycerol levels and the organ blood flow rates of fasting obese subjects. They calculated the total glycerol utilization in these major sites of glycerol metabolism to be 19 g per 24 h. Thus, the 21 g of glycerol found in the urine of our patient during fasting agrees generally with these quantitative observations for the daily free glycerol production in non-hyperglycerolemic subjects. In view of the fact that fasting for 24 h did not appreciably change the creatinine clearance nor the plasma glycerol level, it can be assumed that the increase in urine glycerol which occurred during the fasting period was a result of the increased adipose tissue lipolysis which occurs in fasting (1, 4, 5). The lower urine glycerol content in the non-fasting state can be explained on the basis of the well-known suppression of adipose tissue lipolysis by carbohydrate intake. The declines in the serum levels of non-esterified fatty acid and β-hydroxybutyrate after glucose administration (Table II) provide additional evidence that adipose tissue lipolysis is readily suppressed by carbohydrate in this patient. These findings support the conclusion that the urine glycerol of this hyperglycerolemic subject represents the entire endogenous glycerol production and that there is a virtual absence of glycerol utilization in the tissues.

During the fasting period the patient experienced no symptoms of hypoglycemia and the blood glucose level fell to only 80 mg/dl at the termination of the fast (Table IV). Presuming that the patient is unable to utilize free glycerol for gluconeogenesis, the maintenance of a normal blood glucose level during the 24-h fast indicates that the other substrates of gluconeogenesis (amino acids and lactate) were able to provide adequate substrate for glucose formation.

The observation of an absence of [^{14}C]glycerol oxidation to $^{14}CO_2$ by the isolated leukocytes of the patient together with the finding of a failure of these cells to phosphorylate glycerol and an absence of detectable

levels of leukocyte glycerokinase activity all support the conclusion that the tissues of the patient are unable to metabolise glycerol, and that this is due to impairment in glycerol phosphorylation, the first step of its metabolism (24). The major organs of glycerol utilization are liver and kidney, and these contain high levels of glycerophosphokinase (25). Biopsies of liver or kidney for the purpose of direct observation of glycerol metabolism in the patient's tissues have not been performed.

From the data of Table IV, the average creatinine clearance of the patient is 56 ml/min. At an average plasma glycerol level of 75 mg/dl, the filtered load of glycerol is thus about 60.5 g/24 h. The daily (non-fasting) glycerol content of the urine was about 13 g, and this represents about 20% of the filtered load. These calculations indicate that the renal tubules of the patient are able to reabsorb about 48 g of glycerol per 24 h. If, as is postulated, the patient's tissues are lacking in glycerokinase, the renal tubular reabsorption of glycerol must occur by a mechanism other than phosphorylation. This conclusion is supported by the findings of Sveinsson (26) who studied the renal mechanism of glycerol excretion after intravenous infusion in man and of Kruhøffer and Nissen (27) who performed similar studies in cats. These workers found that metabolic conversion of glycerol by the renal tubules was of significance for tubular reabsorption only at low plasma levels (below 10 mg/dl), whereas at high plasma levels transcellular back-diffusion through proximal tubular cells was more significant. As mentioned above, fasting increases glycerol production from adipose tissue, and this is undoubtedly the source of the increased urine glycerol (ca. 9 g) that was present during the 24-h fast.

The 21-yr-old grandson of the propositus demonstrated a plasma glycerol level of 30 mg/dl and a creatinine clearance rate of 93 ml/min. The daily glycerol loss (non-fasting) was 16 g which indicates that he likely has the same degree of impairment in glycerol utilization as does his grandfather. We have verified this by incubating leukocytes from the affected grandson with [U-^{14}C]glycerol. A total absence of $^{14}CO_2$ production was observed. The marked difference in the plasma glycerol levels of these two subjects is therefore mainly attributable to their differing glomerular filtration rates. This suggests that renal function is the primary determinant of the plasma glycerol level in familial hyperglycerolemia.

Another feature of this condition is the presence of increased serum osmolality without deviation in the levels of electrolytes (Table I). The increased osmolality is undoubtedly partly a result of the high plasma glycerol level which at a level of 75 mg/dl would contribute 8 mosmol. The absence of any apparent physiological response to the increased osmolality in the form of excessive thirst or evidence of increased output of antidiuretic hormone is explainable by the ready diffusion of glycerol across cell membranes, including those of the central nervous system (28). This would normalize the osmotic pressure differential between the intra- and the extracellular fluid compartments and obviate a physiological response for water retention.

The daily loss of 13 g of glycerol represents a loss of approximately 55 calories per day for which dietary compensation is readily made. The fact that this patient has survived 70 yr indicates that this inborn defect of glycerol metabolism is nonlethal and presumably innocuous. Further study will be required to explore the possible relation of the defect to the high incidence of diabetes mellitus in this family.

Because serum triglyceride levels are now commonly measured enzymatically, the occurrence of familial hyperglycerolemia is of practical importance as it could lead to serious over-estimation of the serum triglyceride level. We are however unaware of the occurrence of this type of defect in any family other than the one reported here, but the possibility of this diagnosis should be considered in any case where an elevated serum triglyceride level is found in the absence of corresponding increase in chylomicrons and or pre-β-lipoproteins.

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