THE METABOLISM OF CLOFIBRATE IN MAN

BY

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INTRODUCTION

Atherosclerosis is a common disease of man in the western hemisphere. The complications of atherosclerosis which include ischaemic heart disease, cerebrovascular disease and peripheral vascular disease are responsible for a major proportion of the mortality and morbidity in the population. Finland, Scotland and the United States are among the countries with the world's highest incidence of coronary atherosclerosis in men aged 45-55 years (1). In the United States atherosclerotic cardiovascular disease accounts for the greatest number of deaths from any cause (45% of all deaths in 1967, compared to 16.8% for cancer, 6.1% for accidents and 32.1% for all other causes) (2).

Many studies conducted over the past 30 years have demonstrated associations between certain biochemical, physiological and environmental factors and the development of premature atherosclerosis. Risk factors for atherosclerotic diseases include hyperlipidaemia (3), hypertension (4,5), cigarette smoking (1,5), diabetes mellitus (1) and obesity (1,5).

Primary hyperlipidaemia, that is hyperlipidaemia unassociated with diseases such as hypothyroidism, biliary cirrhosis and the nephrotic syndrome, affects

a substantial proportion of the general population (3). In an effort to minimize the likelihood of developing premature atherosclerosis hyperlipidaemic patients are often treated by dietary means (6,7) and drugs to reduce plasma lipid levels.

Clofibrate (Fig.1), which is the ethyl ester of p-chlorophenoxyisobutyric acid (CPIB) is a widely used hypolipidaemic agent. This drug was introduced in 1962 and has since been established as an effective and relatively non-toxic agent (8,9). Although there have been extensive investigations into the possible modes of action of clofibrate, relatively little work has been done on the pharmacology and metabolism of the drug. The present study was undertaken in an effort to elucidate some of the outstanding problems in this field. The questions addressed were whether cholestyramine or neomycin given with clofibrate interfered with its metabolism, whether patients on long-term clofibrate therapy accumlate the drug and whether this was related to any toxic manifestations of the agent, whether CPIB was degraded in vivo, the situation regarding absorption, routes of excretion and the presence or absence of clofibrate in bile and tissues other than the extracellular fluid compartment of the body.

The first objective was to devise a method for the accurate measurement of CPIB in biological fluids

and the first part of this thesis describes in detail the technique developed for that purpose. Having been satisfied with the accuracy and reliability of the method, a study of the pharmacology and pharmacokinetics of clofibrate in man was undertaken. The second part of the thesis deals with this aspect of the investigation. Fifteen adult patients on 1 g clofibrate twice daily for periods of 2-416 weeks were studied. The results show that clofibrate can be given with cholestyramine or neomycin without any interference with its absorption. Orally administered clofibrate is almost totally absorbed, and thus faecal excretion is minimal. Clofibrate is present in plasma entirely in the form of CPIB. The mean fasting plasma CPIB concentration was 123 µgm/ml, the major portion being albumin bound. No conjugated CPIB was detectable in the plasma. The urine was the major route of excretion of CPIB, 61% of which was conjugated. Conjugation occurred mainly in the kidneys although conjugated CPIB was also present in bile. The mean half life of the drug was 15 hours. Pool size measurements indicated that there was no accumulation of the drug in the body of patients on long-term clofibrate therapy. Although no CPIB was detectable in adipose tissue the drug is probably present in body tissues other than the extracellular fluid compartment.

PART I

DETERMINATION OF CLOFIBRATE IN BIOLOGICAL FLUIDS USING THIN-LAYER AND GAS-LIQUID CHROMATOGRAPHY

A method is described for the isolation and quantification of clofibrate as the acid p-chlorophenoxyisobutyric acid (CPIB) in plasma, urine, bile, adipocytes and faeces, employing thin-layer and gas-liquid chromatography.

Two procedures for the determination of clofibrate in biological fluids have already been published. The method of Thorp (10) utilizes spectrophotometry, whereas that of Horning et al (11) involves gas-liquid chromatography (GLC); recoveries by the former method are known to be incomplete for plasma, and in the latter report the question is not addressed. The method developed in this laboratory utilizes thin-layer chromatography (TLC) to separate CPIB from other fatty acids and depends for its quantitative aspects on the use of an internal recovery standard p-chlorophenoxypropionic acid (CPP), and a GLC standard arachidic acid (20:0). The structural formulae of these two compounds are illustrated in Fig.2. Quantification is by GLC of the methyl esters of CPIB, CPP and 20:0, with detection by hydrogen flame ionisation. The method is specific, sensitive and reproducible, and overall recoveries of CPIB from biological materials are high (>93% for plasma and urine and >84% for bile and faeces).

MATERIALS AND METHODS

Standards

The internal recovery standard p-chlorophenoxy-propionic acid (CPP) was obtained from Pfalz and Bauer, Inc., Flushing, N.Y. The acid was methylated with 5% HCl-methanol and the methyl (Me) ester was purified by TLC using Silica Gel H and benzene. The standard solution of Me CPP contained 260 µg per 200 µl.

The GLC standard, arachidic acid methyl ester (Me 20:0), was obtained from Schwarz-Mann, Orangeburg, N.Y. The standard solution contained 140 µg per 200 µl.

Thin-layer chromatography was carried out on 0.5 mm layers of Silica Gel H (E.M. Reagents, Div. Brinkmann Instruments, Inc., Westbury, N.Y.) on 20 x 20 cm plates. Runs were uniformly developed in toluene-hexane (80:20, V/V), and recoveries were made with diethyl ether as eluent.

Gas-liquid chromatography was carried out on an instrument equipped with a hydrogen flame ionisation detector (F and M Biomedical Gas Chromatograph, Model 400, Avondale, Pa.). A 6 ft. column, 4 mm internal diameter was packed with 10% EGSSX on Gas Chrom Q (Applied Science Laboratories, Inc., State College, Pa.). Nitrogen was used as carrier gas at a

flow rate of 24 ml/min with an inlet pressure of 40 pounds per square inch. The temperatures were: column 180°C., flash heater 220°C., detector 240°C. An electronic integrator was used for quantification of peak areas (Digital Readout Model CRS-10 HB Infotronics, Houston, Texas).

Radioactivity measurements were performed with a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3380). Counting efficiencies were approximately 40% for H^3 and 52% for ^{14}C. Measurements were done in PPO-POPOP-toluene solution prepared from Liquifluor (Pilot Chemicals, Watertown, Mass.). Direct radioactivity counting of plasma (250 ul) and urine (0.5 ml) was carried out with Aquasol (New England Nuclear, Boston, Mass.). Measurements of radioactivity in bile and faeces were made only after extraction. Quenching corrections were performed automatically by an Absolute Activity Analyzer (Packard Instruments, Model 544).

Procedure

1. Saponification

One ml plasma, urine or bile, a known mass of isolated adipocytes from adipose tissue or 1 gm of faeces was mixed with 10 ml methanol in a 125 ml glass stoppered bottle and 200 ul of the internal recovery standard, Me CPP, was added. This mixture was refluxed for one hour

with 1 ml of 10 N NaOH in order to hydrolyse whatever conjugated CPIB might be present. Alkaline hydrolysis was chosen for this purpose because in a separate study it was shown that the recovery of CPIB from the urine of patients on clofibrate was more complete following alkaline than acid hydrolysis.

2. Extraction

After cooling, the mixture was acidified with 1 ml conc. HCl. Twenty ml chloroform and 30 ml chloroform-methanol (2:1, V/V) were then added successively, and the mixture shaken for one minute; 10 ml water was added and the shaking continued for a further minute. Following centrifugation (5 min. at 1000 x g) the lower phase containing CPIB and CPP was transferred to a 500 ml round bottom flask. Twenty ml chloroform was added to the upper phase and the procedure repeated twice in order to assure a quantitative transfer. The solvent was evaporated to dryness with a rotary evaporator.

3. Methylation

Five ml 5% HCl-methanol was added to the dried residue and the mixture left overnight; the methyl esters were concentrated by rotary evaporation. Because of the volatility of Me

CPIB and Me CPP, the evaporation must be carried out at room temperature; care must be taken to stop the evaporation as soon as the residue appears dry.

4. Thin-Layer Chromatography

Me CPIB, Me CPP and methylated fatty acids were quantitatively transferred with chloroform-methanol (2:1) to TLC plates. Because of the large amount of fatty acids in plasma, bile, adipocytes and faeces, one TLC plate is required for each sample in order to obtain a clean separation of the Me CPIB and Me CPP from the fatty acids. A standard mixture of Me CPIB and Me CPP was applied at one side of the plate. The plates were run in toluene-hexane (80:20), and the solutes were visualised with iodine vapour. Me CPIB and Me CPP run together (R_f 0.41), separated from other fatty acids (R_f>0.47, Table 1). The portion of the plate corresponding to the standard mixture was scraped off and eluted with 4 x 4 ml diethyl ether into a 100 ml round bottom flask. At this stage 200 µl of Me 20:0, the GLC internal standard, was added. The mixture was again cautiously evaporated to dryness and redissolved in 1 ml methanol. Radioactivity was counted on a 0.5 ml aliquot, when appropriate, and the rest utilized for GLC.

5. Gas-Liquid Chromatography

Five µl of the sample was used for GLC. The first peak in the chromatogram is that of Me CPIB with a relative retention time (RRT) to Me 20:0 of 0.55. The internal recovery standard Me CPP appears at a RRT of 0.75 (Fig.3).

Table 2 relates the hydrogen flame ionisation responses of Me CPIB, Me CPP and Me 20:0. The area response of Me CPIB is almost identical to that of Me CPP. However, the area response of Me CPP is only 63% that of Me 20:0. This requires a correction factor of 100/63 to be applied when calculating Me CPP recovery relative to that of Me 20:0. This factor has been verified by several other experiments in which the proportions of the three constituents were widely varied.

6. Calculation

The amount of CPIB in the sample is calculated in terms of the CPP recovery standard as follows:

µg CPIB in 1 ml plasma = (Me CPIB area response/Me CPP area response) x 260

The recovery of Me CPIB (and/or Me CPP) is calculated as follows, utilizing the correction factor of 100/63:

% recovery of Me CPIB (= % recovery of Me CPP) =

(Me CPP area response/Me 20:0 area response) x 140 x $\frac{100}{63}$ x $\frac{100}{260}$

RESULTS

1. Suitability of the internal recovery standard

Losses of CPIB occur at various stages in this procedure, necessitating the use of the overall recovery standard CPP. Up to the methylation step the drug is in the form of the free acid CPIB (m.p. 120°C) which is much less likely to be lost during the extraction procedure than is the more volatile Me CPIB (boiling point 138°C at 18 mm Hg) during and after TLC. Using plasma containing ^{3}H-CPIB, losses amounted to 1.2% up to the TLC step. During and after TLC, losses of both Me CPIB and Me CPP occur by evaporation, but as seen in Table 3 these losses are almost identical: rotary evaporation at 25°C is clearly preferable to use of a nitrogen stream at that temperature.

2. Specificity

During TLC, Me CPIB is usually cleanly separated from the other fatty acids present

in plasma, bile and faeces. Occasionally however, small amounts of fatty acids are eluted with Me CPIB and Me CPP and appear in the final sample analyzed by GLC. The GLC peaks of the methyl ester of myristic acid (Me 14:0), the methyl ester of palmitic acid (Me 16:0) and the methyl ester of palmitoleic acid (Me 16:1) appear before the peak for Me CPIB and in no way interfere with its quantification. However, the RRT of the methyl ester of stearic acid (Me 18:0) which is 0.58 is so close to that of Me CPIB (RRT 0.55) that isolation of Me CPIB from fatty acid methyl esters by TLC must be complete in order to accurately estimate CPIB. To confirm that TLC can completely separate these two groups of compounds, ^{14}C-labelled stearic acid (18:0) was added to plasma containing ^{3}H-labelled CPIB prior to analysis. The results are shown in Table 4. None of the ^{3}H-labelled CPIB was recovered in the "fatty acid fraction", and 3% or less of the ^{14}C-labelled 18:0 was recovered in the "drug fraction". Thus, even though these data demonstrate that fatty acids can successfully be separated from CPIB and CPP by TLC, the operational rule should be adhered to that, when the GLC pattern indicates a significant contamination of the pattern by

fatty acid methyl esters, the sample should be subjected to repeat TLC in which only the trailing part containing the drug is recovered. Significant contamination is indicated by the finding of measurable peaks other than those of the methyl esters of CPIB, CPP and 20:0.

3. Sensitivity

Assuming 80% recovery and the utilization of 5 µl of sample for GLC, the method as described detects levels of CPIB less than 10 µg per ml of plasma, urine or bile or per g of faeces. If smaller quantities of the drug are anticipated, decreasing the volume of methanol added at the end of the procedure to 0.5 ml will increase detection sensitivity to less than 5 µg per ml. If radioactivity counting is not required, solution of the final mixture of methyl esters can be carried out with as little as 10 µl methanol in which case the sensitivity is increased to 0.1 µg per ml.

4. Recovery and Precision

To determine the actual recovery of CPIB from biological fluids (uncorrected by use of the internal recovery standard CPP), two approaches were used. The first involved the addition of unlabelled clofibrate in the form of the free acid CPIB to plasma and urine. The results shown in Table 5 indicate that the

recovery from plasma was 94% and from urine 93%. Similar tests for recoveries from faeces were 85% (range 80-91%) and from bile 84% (range 83-85%).

In the second approach plasma and urine from a subject given ^{3}H-labelled clofibrate by mouth was used. The recovery of CPIB was calculated by comparing the total counts in plasma and urine with those obtained after extraction and isolation of the drug by the procedure described above. The results are shown in Table 6. Recovery of labelled CPIB from plasma was 92% and from urine 91%.

Reproducibility of the method was assessed by running 8 sets of replicate samples. Table 5 shows that the mean coefficient of variation was 6% with a range of 2-11%.

DISCUSSION

The method described can be utilized for the specific detection of CPIB and its accurate quantification in plasma, urine, bile, stool and adipose tissue. We have not applied it for analyses in other tissues.

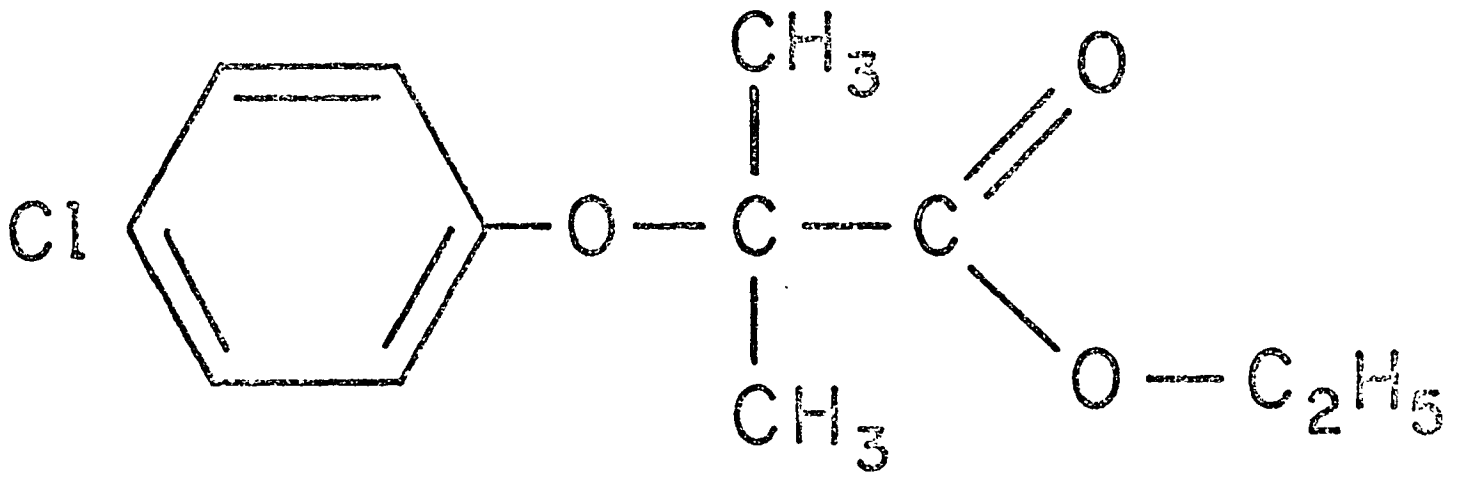
In patients on long-term clofibrate therapy (daily oral dose of 2 gm) considerable quantities

of CPIB are present in the urine and moderate amounts in the faeces. Fasting plasma levels of CPIB are usually greater than 100 µg per ml, and fasting biliary levels are greater than 50 µg per ml (12). As the method can detect CPIB levels as low as 0.1 µg per ml, its sensitivity appears to be satisfactory for further pharmacokinetic studies.

Recovery of CPIB is greater than 90% from plasma and urine, and greater than 80% from bile and faeces. But whatever the losses, the properties of the internal recovery standard, Me CPP, are such that a precise correction can be made in the final calculation of Me CPIB. This feature of the method suggests that "shortcuts" can be usefully applied, such as a single extraction of the acidified saponification mixture with chloroform-methanol 2:1 (88% recovery from plasma), or single transfer of the methyl ester mixture to the TLC plate (79% recovery from plasma). Although final yields are reduced, the internal recovery standard compensates adequately. Although not used in these tests, more rapid methods of methylation may be useful, such as diazomethane, 2,2-dimethoxypropane or methanol containing 5-10% boron trifluoride.

The assay of clofibrate in biological fluids as carried out by the spectrophotometric technique devised by Thorp (10) is stated to give excellent

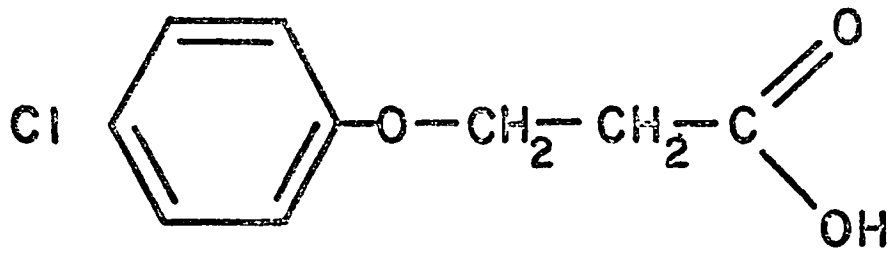
recovery from urine but only 68% recovery from serum and tissues. We have verified that the Thorp method is nearly quantitative for urine but in our hands the values for plasma are 40-45% those determined by the present procedure. Thus, the method described in this report improves on recovery from serum, introduces a reliable internal recovery standard to correct for losses during the procedure, and depends for final analysis on the more specific method of GLC. One operator can complete the analysis of 32 samples in 5 days by this approach. The less specific spectrophotometric method of Thorp allows a five-fold greater accumulation of data and hence is to be preferred as a screening procedure. The present method offers advantages, however, for accurate studies of drug metabolism.



Clofibrate

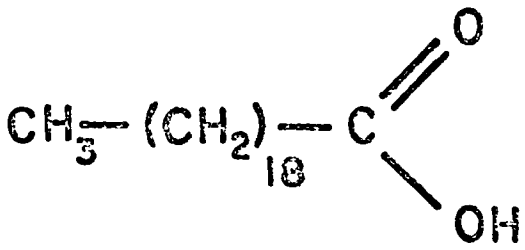
(Ethyl ester of CPBI)

Figure 1. The structural formula of the ethyl ester of p-chlorophenoxyisobutyric acid (Clofibrate).



p-Chlorophenoxypropionic Acid

(CPP)



Arachidic Acid

(20:0)

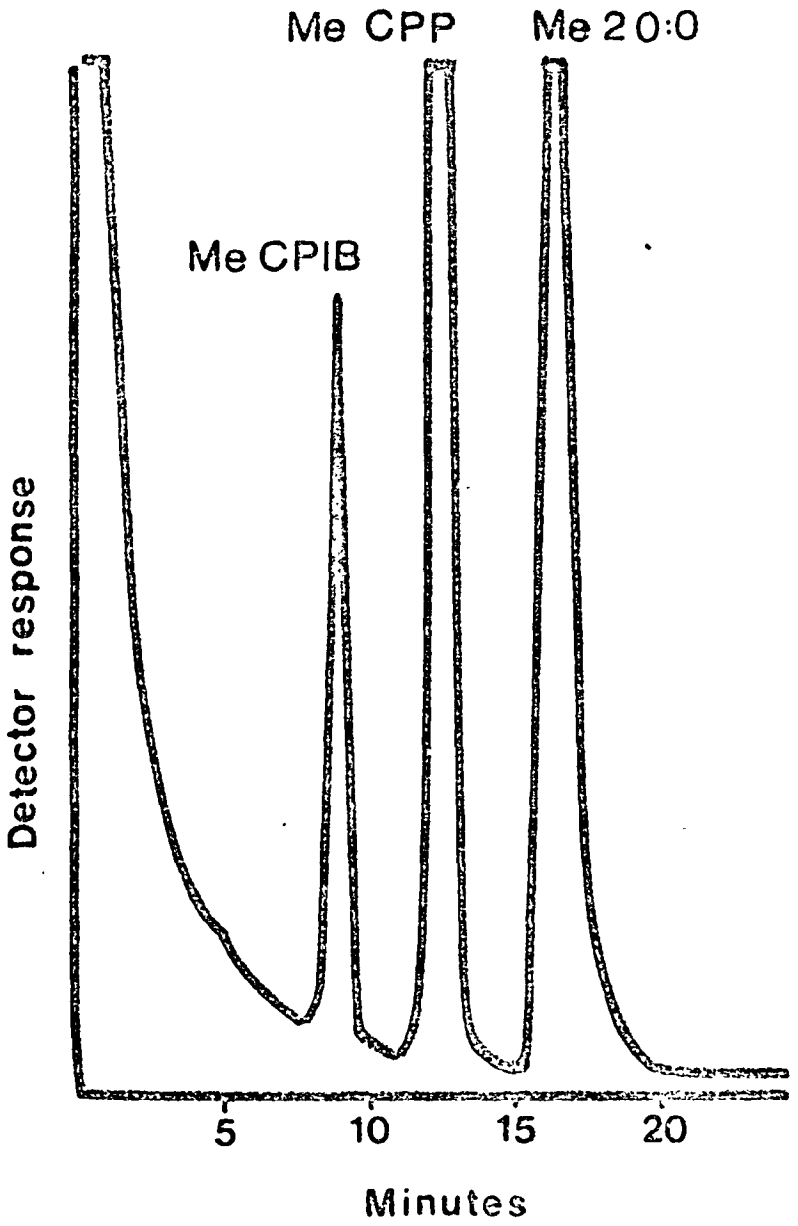
Figure 2. The structural formulae of the internal recovery standard p-chlorophenoxypropionic acid and the GLC standard arachidic acid.

TABLE 1

R_f Values and Relative Retention Times of the Methyl Esters of Plasma Fatty Acids, CPIB, CPP and 20:0

Compound		TLC R_f Value	GLC Relative Retention Time
Methyl myristate	(Me 14:0)	0.5	0.20
Methyl palmitate	(Me 16:0)	0.5	0.35
Methyl palmitoleate	(Me 16:1)	0.5	0.43
Methyl Stearate	(Me 18:0)	0.53	0.58
Methyl oleate	(Me 18:1)	0.5	0.71
Methyl linoleate	(Me 18:2)	0.47	0.91
Methyl ester of CPIB	(Me CPIB)	0.41	0.55
Methyl ester of CPP	(Me CPP)	0.41	0.75
Methyl arachidate	(Me 20:0)	0.5	1.00

R_f values (relative to the solvent front) were obtained by running the samples on Silica Gel H plates in toluene hexane (80:20). The GLC retention times are relative to Me 20:0, using 10% EGSSX on Gas Chrom. Q.



Gas chromatographic analysis of the methyl esters of CPIB CPP and 20:0

Figure 3

TABLE 2

Hydrogen Flame Ionisation Response of Me CPIB, Me CPP and Me 20:0

GLC injection mixture (µg)		Me CPIB Calculated (µg)	Hydrogen flame ionisation response of equal masses of Me CPIB, and Me CPP (%)
Me CPIB	Me CPP		
339.9	297.4	343.6	101
283.2	297.4	285	101
226.6	297.4	225	99
170	297.4	166.4	98
113.3	297.4	108.7	96
56.7	297.4	56.4	100

GLC injection mixture (µg)		Me CPP Calculated (µg)	Hydrogen flame ionisation response of equal masses of Me CPP and Me 20:0 (%)
Me CPP	Me 20:0		
446.2	145.8	281	63
390	145.8	261.3	67
260	145.8	156	61
195	145.8	122.9	61
149	145.8	90.8	63
130	145.8	81.9	63

TABLE 3

Recovery of Me CPIB and Me CPP Following Evaporation to Dryness of a Mixture under Different Conditions*

Conditions of Evaporation	Recovery %		Ratio
	Me CPIB	Me CPP	Me CPIB/Me CPP
Vacuum at 25°C	100	97	1.03
	101	100	1.01
Vacuum at 40°C	97	93	1.03
	79	72	1.09
Vacuum at 50°C	73	70	1.03
Nitrogen stream at 25°C	63	61	1.05
	87	86	1.01
Nitrogen stream at 40°C	18	20	0.98
	15	17	0.88

* The recovery of Me CPIB and Me CPP was computed by GLC, using Me 20:0 as recovery standard.

TABLE 4

Separation of Me CPIB from Me 18:0 during TLC Step. Tritiated Me CPIB and ^{14}C Labelled Me 18:0 were used to estimate Completeness of Separation

	% ^{3}H Me CPIB		% ^{14}C Me 18:0	
Plasma Samples	Drug Fraction*	Fatty Acid Fraction	Drug Fraction	Fatty Acid Fraction
1	100	0	1.8	98.2
2	100	0	3	97
3	100	0	1.6	98.4
4	100	0	1.3	98.7

* Drug fraction refers to portion of TLC plate corresponding to Me CPIB and Me CPP standard run on one side.

TABLE 5

Recovery of Unlabelled CPIB from Plasma

CPIB Added to 1 ml Plasma (µg)	53.68	107.36	161	214.72
% Recovery of Added CPIB	93	84	99	94
	89	101	88	97
	90	95	100	85
	112	103	88	86
Average ± S.D.	96 ± 11	96 ± 9	94 ± 7	91 ± 6
		Mean Recovery 94%		
Coefficient of Variation (%)	11	9	7	7

Recovery of Unlabelled CPIB from Urine

CPIB Added to 1 ml Urine (µg)	53.68	107.36	214.72	322
% Recovery of Added CPIB	84	92	95	88
	83	90	97	98
	84	89	100	103
	89	94	90	104
Average ± S.D.	85 ± 3	91 ± 2	96 ± 4	98 ± 7
		Mean Recovery 93%		
Coefficient of Variation (%)	4	2	4	7

TABLE 6

Recovery of Tritiated CPIB from Plasma

DPM per ml Plasma

Direct Count	Count at End of Isolation Procedure	Recovery %
3204	2942	92
3100	2752	89
3372	3178	94
3372	3032	90
2720	2748	101
2720	2662	98
3372	2858	85
3372	2876	85
	Mean ± S.D.	92 ± 6

Recovery of Tritiated CPIB from Urine

DPM per ml Urine

Direct Count	Count at End of Isolation Procedure	Recovery %
9792	8806	90
9792	8458	86
10406	9398	90
10406	9616	92
10406	9524	92
10406	8888	85
10406	9968	96
10406	9928	95
	Mean ± S.D.	91 ± 4

PART II

THE METABOLISM OF CLOFIBRATE IN MAN

MATERIALS AND METHODS

Patients

Studies were carried out on 15 patients whose age, sex, body weight and clinical diagnosis are presented in Table 1. There were 5 male and 10 females aged 17-72 years. Patients 1-8 were studied during hospitalisation on a metabolic ward at The Rockefeller University Hospital; patients 9 and 10 were studied as outpatients. Patients 11-15 were hospitalised at the Mount Sinai Hospital under the care of Dr. D. Pertsemlidis. These patients had undergone cholecystectomy for cholelithiasis. Bile samples from these patients were obtained via a common bile duct catheter inserted at the time of operation.

Diets

All patients were on solid food except patients 7 and 8 who were given liquid formula feedings of known composition for independent sterol balance studies.

Drugs

Clofibrate in a dose of 1 g twice daily was administered to all patients with the exception of patient 7 who received 500 mg of the drug three times daily. Clofibrate was administered in the form of the commercial preparation, Atromid-S (Ayerst Co., New York). On the basis of the molecular weights of clofibrate and CPIB (243 and 215, respectively), each

500 mg capsule contained 442 mg of CPIB. All patients had been on continuous clofibrate therapy for 2-416 weeks prior to the commencement of these studies. In addition, cholestyramine was administered to patients 1-4, 7 and 8 in a dose of 4 g 4 times daily for periods of 10-14 days. The first and last doses of cholestyramine were given at the same time as clofibrate. Neomycin (1 g twice daily) was administered to patient 2 for 4 weeks. Other than the above, no drugs were being taken by the patients in this study.

Isotopic materials

Randomly tritiated clofibrate of specific activity 14.6 μCi/mg was obtained from Sandoz Pharmaceuticals, Hanover, New Jersey. ^{14}C-labelled clofibrate was generously provided by Dr. J.M. Thorp, I.C.I., Macclesfield, England. This compound was labelled in the side chain (Fig.1) and had a specific activity of 7.30 μCi/mg.

The radioactive compounds were purified by thin-layer chromatography on Silica Gel H; the plates were developed in benzene and only the material that chromatographed with the same R_f as the pure clofibrate standard was extracted. Following saponification, the free acid p-chlorophenoxyisobutyric acid, was extracted with chloroform methanol (2:1), evaporated to dryness and redissolved in ethanol.

For oral administration ^{14}C-labelled CPIB (7-10 µCi) was dissolved in ethanol, mixed with fruit juice and given at 9 am.

Tritiated CPIB was administered intravenously; the labelled material in ethanol (4-20 µCi) was mixed with the patients own serum (the final mixture containing less than 0.1% ethanol), passed through a Swinnex filter unit of 0.45 mµ pore size (Millipore Corporation, Bedford, Mass.) and infused intravenously. Residual radioactivity was determined after chloroform methanol (2:1) extraction of the infusion equipment.

To exclude any losses of tritium from the randomly labelled ^{3}H CPIB during the analytical procedures and by in vivo biological exchange, patient 4 was infused with a mixture of ^{3}H CPIB and ^{14}C CPIB intravenously. The stability of tritiated CPIB was confirmed by the finding that the plasma ratio of the two isotopes was constant throughout the duration of the experiment (Table 2).

Analytical procedures

Analysis of biological material (plasma, bile, urine, faeces, etc.) was carried out by a method developed in this laboratory and described in detail in Part I of this thesis. Methyl p-chlorophenoxy-propionate was used as an internal recovery standard to correct for losses of CPIB during the procedure. Radioactivity measurements were performed with a

Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3380).

Quantification of conjugated CPIB in urine and bile

It is believed that a major portion of urinary CPIB is in the conjugated form (11,13). This conjugated fraction of the drug is water-soluble and is not extracted into the chloroform phase when acidified urine is treated with chloroform methanol (2:1). Free CPIB, however, is extracted by this procedure. Initial saponification followed by acidification and chloroform methanol extraction recovers all the CPIB. The difference between total and free CPIB represents the conjugated material.

CPIB in plasma

In an attempt to determine whether conjugated CPIB was present in plasma, the recovery of CPIB from acidified plasma extracted with chloroform methanol (2:1) was compared with CPIB recovered by saponification followed by acidification and chloroform methanol (2:1) extraction.

To confirm that CPIB in plasma is mainly albumin-bound, 200 µl of plasma containing tritiated CPIB was electrophoresed on Whatman No.1 paper in 0.1 M barbital buffer of pH 8.6 for 4 hours. The paper strips were dried at 32°C and some were stained with Amido Black. Good separation of the albumin from the other plasma protein fractions was obtained. Sections of the paper

containing the albumin fraction, the globulin fraction and the site of application were cut off and counted in vials with Aquasol.

Measurements of CPIB absorption

Method I: In this method a known amount of ^{14}C-labelled CPIB was given by mouth and stool collections were carried out for 6-8 days. The amount of labelled CPIB excreted in the faeces in that period was determined and absorption of the drug was considered to represent the difference between the dose given and the amount recovered in the faeces.

Method II: An attempt was made to assess the applicability of measuring CPIB absorption by the "plasma ratio method" devised by Zilversmit to measure cholesterol absorption in the rat (14). This method involves the administration of differently labelled oral and intravenous cholesterol simultaneously and the use of a single blood sample to determine absorption as follows:

plasma specific activity (SA) of orally administered cholesterol / plasma SA of infused cholesterol X dose of infused cholesterol / dose of oral cholesterol X 100. In other words plasma isotope ratio X 100 adjusted for differences in radioactivity of oral and intravenous doses.

Patients were given intravenous ^{3}H CPIB 1-4 hours following the oral administration of ^{14}C CPIB.

Blood samples were taken at regular intervals for up to 24-96 hours after the oral dose had been given so that absorption could be measured several times in each patient.

Adipose tissue analysis for CPIB

To determine whether CPIB was present in tissues other than the extracellular fluid compartment of the body in patients on long-term clofibrate therapy, adipose tissue biopsies were performed in several patients. Some of these patients were infused with ^{3}H CPIB 2 hours prior to the procedure. Adipocytes uncontaminated with CPIB containing interstitial fluid were obtained by the following technique: the skin of the buttock was infiltrated with 1% xylocaine and a size 15 needle attached to an airtight 50 ml plastic syringe was introduced into the adipose tissue raised by piching up a large fold of skin. With suction applied to the syringe the needle was moved to and fro among the adipose tissue. The shreds of adipose tissue sucked up into the syringe were washed several times with saline, blotted dry and suspended in 3 ml Krebs Ringer bicarbonate buffer containing 1 mg collagenase. After incubation at $37^{\circ}C$ for an hour the mixture was filtered and the residue was washed with 15 ml buffer several times. The air dried residue consisting of isolated adipocytes was weighed and then analysed for CPIB including radioactivity counting.

Kinetic studies

To determine the half-life and pool size of CPIB several patients were given ^{3}H CPIB intravenously. Plasma half-life was calculated by plotting disintegrations per minute per ml plasma against time on a semilogarithmic graph. Drug pool size was measured by extrapolating the log-linear portion of the plasma CPIB specific activity time curve to zero time and dividing the administered dose by the specific activity at zero time.

CPIB pool size by mass measurements

To check the validity of measuring CPIB pool size by kinetic analysis use was made of measuring the urinary and faecal excretion of CPIB in 2 patients who had been on long-term clofibrate therapy and whose intake of the drug was stopped at the time these measurements were commenced. The excretion of CPIB in the urine and faeces was measured for 6 days (after which time there was no further appreciable excretion of the drug) and the total was compared with the pool size calculated from isotope kinetic data at the time that urine and stool collections were commenced.

RESULTS

Plasma CPIB

Fasting levels: The mean plasma CPIB concentration measured before the morning dose of clofibrate in 12 patients was 123 µgm/ml, with a range of 82-165 µgm/ml (Table 3). There was a significant correlation between fasting plasma CPIB levels and the dose of the drug as expressed in mg per kg body weight per day (Figure 2).

To achieve a fasting plasma CPIB concentration of 123 µgm/ml, a dosage schedule of 32 mg clofibrate per kg body weight (given in two divided doses) would be required.

Peak levels: In 4 patients who had serial blood samples analysed the mean peak plasma CPIB concentration was 193 µgm/ml, range 165-229 µgm/ml. Peak plasma CPIB levels were achieved 3.5 hours following the intake of 1 g clofibrate with breakfast.

Conjugated CPIB: Plasma CPIB levels in 2 patients as determined by saponification and acidification prior to chloroform methanol extraction did not differ significantly from the values obtained by acidification only prior to extraction (127 and 103 µgm/ml compared to 132 and 100 µgm/ml). Although the method utilised in this study to measure CPIB concentrations is fairly sensitive, it may not have been sensitive enough to detect small amounts of CPIB conjugate which may have

been present in the plasma. However, plasma obtained from a patient given labelled CPIB by mouth showed insignificant amounts of radioactivity in the supernatant following acidification and extraction with chloroform methanol. (In several runs Aquasol radioactivity counting of the supernatant, which would contain any conjugated CPIB, averaged 0.12% of the total). These findings indicate that virtually no conjugated CPIB is present in the plasma.

Enzymes in liver microsomes are usually involved in the hydrolysis as well as the oxidation and reduction of foreign compounds. These reactions may occur to a lesser degree in other parts of the body such as the gastrointestinal tract and the kidney. In view of the fact that clofibrate given by mouth appears in the plasma almost entirely in its hydrolysed form (p-chlorophenoxyisobutyric acid), it seems probable that hydrolysis of this compound occurs in the gastrointestinal tissue or the liver or both.

Albumin binding of plasma CPIB: Figure 3 shows that almost all the labelled CPIB in plasma (93%) moved with the albumin fraction on paper electrophoresis. This indicates that the major fraction of plasma CPIB is albumin bound.

Biliary CPIB

The mean biliary CPIB concentration measured in 5 patients before their morning dose of clofibrate with

breakfast was 55 µgm/ml, range 20-89 µgm/ml (Table 4). Conjugated CPIB accounted for 40% of the total. There was a significant correlation between fasting biliary CPIB levels and fasting plasma CPIB concentrations ($r = 0.8569$, $p < 0.005$).

CPIB absorption

Absorption of CPIB in patients 2, 5 and 6, using Method I was almost complete (mean 99.6%). Absorption calculated according to Method II, however, differed considerably from Method I, the mean being 76% in patient 2, 116% in patient 5 and 117% in patient 6 (Table 5).

Absorption of CPIB by Method I in patient 2 (99.83%) compares favourably with the daily faecal excretion of administered CPIB (2%). This was the only patient in whom simultaneous absorption and faecal excretion measurements were made.

Faecal excretion of CPIB

Because of the almost complete absorption of CPIB, faecal excretion of the drug was minimal. Patient 2 excreted 39 mg, patient 7 excreted 140 mg and patient 8 excreted 81 mg of CPIB in the faeces per day (Table 6). These values represent 2, 10 and 5% of the daily CPIB intake. The relatively high faecal excretion in patient 7 may have been secondary to her ileal resection which had been carried out some time previously for severe hypercholesterolaemia.

Excretion of CPIB in the urine

The mean 24 hour urinary excretion of CPIB, measured in 7 patients, was 1811 mg (Table 7). This is 102% of the daily CPIB intake. Conjugated CPIB accounted for 61% of the total. There was a significant correlation between fasting plasma CPIB concentrations and renal function expressed in terms of the creatinine clearance ($r = -0.9602$, $p = < 0.005$). In view of the fact that almost all the drug is excreted by the kidneys this correlation would not be unexpected. However, as will be seen later, this correlation is probably fortuitous.

Patients 5 and 6 who were given ^{14}C labelled CPIB orally excreted 100% and 99% of the administered dose in the urine within 6 days. These findings not only indicate that the urine is the major route of excretion of absorbed CPIB, but also that in vivo degradation of the drug does not occur.

CPIB content of adipose tissue

No CPIB was detected in the adipose tissue of patients 1, 2 and 4 (Table 8). In patient 3, 12 µgm of CPIB was present in 253 mg of adipocytes. However, because of some difficulty with the processing of this patient's adipocytes it is possible that the fat cells were contaminated with CPIB containing interstitial fluid.

Patients 2-4 who had been infused intravenously with ^{3}H CPIB 2-3 hours prior to the adipose tissue biopsy had negligible counts in the material analysed.

Kinetic data

Half-life: The mean half-life of plasma CPIB in patients 2-6 according to isotope kinetic data was 15 hours (Table 9). In patients 2 and 6 the half-life of the drug calculated from the plasma CPIB concentration curve correlated well with the half-life obtained from the kinetic data.

Although there was significant negative correlation between creatinine clearance and fasting plasma CPIB concentrations, implying elevated blood levels of CPIB in the face of compromised renal function, we were unable to demonstrate a significant correlation between creatinine clearance and the half-life of plasma CPIB ($r = 0.0707$). Neither were we able to show a significant correlation between plasma CPIB concentrations and plasma CPIB half-life ($r = 0.1766$). These findings imply that the correlation between creatinine clearance and fasting plasma CBIB levels is fortuitous; and indeed on further scrutiny it became apparent that this was so as patients with compromised renal function happened to be receiving the largest dose of clofibrate in terms of mg per kg body weight per day thus accounting for the high blood CPIB levels in these patients.

Pool size: The mean pool size of CPIB in patients 2-4 was 1560 mg (Table 9). These patients were infused intravenously with ^{3}H labelled CPIB 3 hours

following the morning dose of clofibrate. The timing was so designed because it was felt that absorption would be complete by 3 hours and that the largest pool size would be measured. Figure 4 shows the plasma CPIB specific activity-time curve in patient 2, it being representative of all the patients studied. The curve becomes log linear an hour after the infusion of ^{3}H CPIB and remains horizontal. The plasma CPIB specific activity-time curve is horizontal because once the labelled CPIB has mixed with the body pool of the drug, both are handled similarly by the process of excretion. In the absence of absorption of unlabelled drug from the gut, the specific activity (dpm/mg) remains constant with time. Absorption of an oral dose of unlabelled CPIB results in a decrease in the plasma CPIB specific activity (Figure 5).

The pool size of the drug was measured by extrapolating the horizontal log linear portion of the specific activity-time curve to zero time (t_o) and dividing the dose of administered ^{3}H CPIB by the specific activity at t_o.

The calculation of pool size in this manner should give results greater than the true value because first, the radioactivity administered at t_o would be greater than that present at the moment the curve becomes log linear, and secondly, the extrapolated specific activity at t_o is smaller than it would have been had

mixing of the labelled CPIB with the body pool occurred immediately.

To check the validity of calculating pool size in this manner patients 5 and 6 had their pool sizes calculated by mass measurements as well. These measurements were commenced 3 hours after breakfast with the patients missing out their morning dose of clofibrate. The results (Table 9) show a fairly close correlation between the pool sizes obtained in these two different ways.

The initial non-horizontal portion of the specific activity-time curve could be explained on the basis of continuing absorption of unlabelled CPIB from the gut (in the case of patients 2-4 who were given their morning dose of the drug) or transfer of labelled CPIB from the plasma to other pools such as the extracellular fluid and other body tissues. The latter must be the explanation for patients 5 and 6 because they were not given their morning dose of clofibrate and therefore could not be absorbing unlabelled CPIB from the gut.

Figures 6 and 7 show the radioactivity counts per ml plasma plotted against time in patients 3 and 4. It will be seen that there is an interval of one hour from the time of infusion to the curve becoming log linear. As this curve is totally independent of the mass of unlabelled CPIB in the body and therefore of continuing absorption of the drug, the initial non-horizontal

portion of the curve must be due to "mixing". Thus the initial non-log linear portion of the specific activity-time curve in patients 2-4 is probably due to mixing and not due to continued absorption of unlabelled material from the gut.

Further information becomes available from the study of these plasma kinetic curves; incoming CPIB mixes rapidly (within an hour) with the body pools of the drug and absorption of CPIB from the gut is completed within 3 hours of taking the drug with breakfast.

The 5 patients in this part of the study had been on continuous clofibrate therapy for between 3 to 416 weeks. There was no significant positive correlation between duration of therapy and drug pool size ($r = -0.9815$), indicating that drug accumulation in patients on long-term treatment is not likely to occur.

Effects of cholestyramine on clofibrate metabolism

The concomitant administration of cholestyramine and clofibrate was not associated with any significant or consistent changes in the parameters of clofibrate metabolism studied. Fasting plasma CPIB concentrations decreased in 3 and increased in 2 of 5 patients (Table 10). The 24 hour urinary excretion of the drug studied in 4 patients showed an increase in 2 and a decrease in the other 2 patients. Faecal excretion of CPIB increased in 2 patients and decreased in the third.

Similar changes were noted in pool size; in two there was a small decrease and in one an appreciable increase (Table 11).

The only consistent finding was that the addition of cholestyramine to the clofibrate regimen resulted in a delay in the appearance of peak plasma CPIB levels in all of the 3 patients by a mean of 2.5 hours, range 1.5-4 hours (Figure 8).

Effects of administering neomycin with clofibrate

The mean fasting plasma CPIB level during 5 weeks of clofibrate therapy in patient 2 was 200 ± 15 µgm/ml (n = 12). During the concomitant administration of neomycin and clofibrate for a period of 4 weeks it was 215 ± 14 µgm/ml (n = 7). The difference was not statistically significant.

DISCUSSION

A major risk factor in the development of premature atherosclerosis is hyperlipidaemia (3). Hyperlipidaemic patients have elevated plasma cholesterol or triglycerides or both. The increase in plasma cholesterol with age (15) should be taken into account when considering the lipid profile of patients with suspected hyperlipidaemia. It is believed that the risk of developing premature atherosclerosis, in particular coronary artery disease, increases as the serum cholesterol level rises (3).

A variety of diseases are associated with hyperlipidaemia and in these situations treatment of the primary disorder results in the return of elevated lipid levels to normal. These diseases include hypothyroidism, the nephrotic syndrome, biliary cirrhosis, diabetes mellitus, alcoholism, pancreatitis and the dysproteinaemias. Primary hyperlipidaemia is said to exist when secondary causes of elevated blood lipids have been excluded by appropriate investigations.

Patients with primary hyperlipidaemia have been classified in a variety of ways. One recent classification (16) employing lipoprotein electrophoresis as well as measurements of fasting plasma cholesterol and triglycerides divides patients with hyperlipidaemia into five types.

The Type I patients have fasting chylomicronaemia with high plasma cholesterol and very high plasma

triglyceride levels. They present as children or young adults with recurrent episodes of abdominal pain. Examination may reveal hepatosplenomegaly. These patients have a deficiency of the enzyme lipoprotein lipase. Type II patients have elevated plasma cholesterol values but may have either normal (II a) or elevated plasma triglycerides (II b). Lipoprotein electrophoresis demonstrates increased amounts of β lipoproteins. Patients with tendon or skin xanthomas probably have the hereditary form of the disease. The gene is autosomal dominant; the homoxygotes have a very poor prognosis, most dying of coronary artery disease before reaching adult life. The Type III patients have elevated plasma cholesterol and triglyceride levels. Lipoprotein electrophoresis demonstrates a broad band connecting the β to the pre β band. Type IV patients have elevated plasma triglyceride levels. Plasma cholesterol may also be elevated. Lipoprotein electrophoresis shows an excess of pre β lipoproteins. The fasting plasma of these patients after overnight incubation at $4^{o}C$ appears cloudy. The condition is often familial and the patients tend to be obese and show glucose intolerance. Type V patients have elevated plasma cholesterol and very high plasma triglyceride levels. Fasting plasma incubated at $4^{o}C$ shows a creamy layer floating on turbid plasma. These patients tend to have recurrent episodes of abdominal pain and may show eruptive xanthomas and lipaemia retinalis.

The objectives of therapy are twofold. Patients with Types I and V hyperlipidaemia have recurrent episodes of abdominal pain which may or may not be related to pancreatitis. Successful treatment of these patients prevents further attacks. Probably all hyperlipidaemic patients (except those with Type I hyperlipoproteinaemia) are at risk of developing premature atherosclerosis. Because of the belief, as yet unconfirmed, that nomalising plasma lipid levels decreases the incidence and severity of atherosclerosis, many workers in this field are of the opinion that an attempt should be made to achieve this goal. Many hyperlipidaemic patients benefit from dietary treatment (17). By reducing dietary fat intake Type I patients achieve considerable improvement in their plasma triglycerides and become asymptomatic. Plasma cholesterol levels may be decreased appreciably in Type II patients by decreasing dietary cholesterol and saturated fats and increasing the intake of unsaturated fats (6,17). Type III and IV patients benefit from weight reduction and Type V patients improve on a diet restricted in both fat and carbohydrates. Type I patients and some of the patients belonging to the other hyperlipidaemic categories can be controlled satisfactorily by dietary means alone. A great many, however, need one or another of a group of hypolipidaemic drugs. The drugs that are currently available for the treatment of hyperlipidaemia include clofibrate, cholestyramine, nicotinic acid, neomycin,

D-thyroxine and para amino salicylic acid. Those most commonly used are clofibrate, cholestyramine and nicotinic acid. Clofibrate is used for the treatment of hyperlipoproteinaemic patients with Types II a, II b, III, IV and V. Cholestyramine is used primarily for the treatment of patients with Type II hyperlipidaemia.

Clofibrate, which is the ethyl ester of parachlorophenoxyisobutyric acid, was found to be effective in reducing the liver and serum content of lipids in rats by Thorp and Waring in 1962 (18). The activity of the drug in the rat was increased during periods of relative adrenocortical hyperfunction and thus its action was thought to enhance the metabolic effects of adrenal steroids. In the rat androsterone given by mouth in combination with CPIB ester was the most effective steroid in reducing serum and liver lipids. Oliver (19) showed that the combination of CPIB with androsterone (Atromid) was effective in lowering serum lipids in man. Further trials, however, showed that androsterone was not necessary and that CPIB ethyl ester alone (Atromid S) was the active hypocholesterolaemic agent (20,21). Since then clofibrate has been used extensively in the management of hyperlipidaemic states.

Adverse effects are infrequent. Nausea has been reported to occur in 4% of patients (22) but this symptom is short lived. Gradual weight gain occurs in some patients (20) but it has been shown that this is not due

to increase in plasma volume or total body water (23). Elevations of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) during Atromid therapy was reported by Oliver in 1962 (19). More recently Langer and Levy (24) reported elevated serum transaminase and creatine phosphokinase (CPK) levels in 5 of 60 patients being treated with clofibrate. Two of the 5 patients had severe myalgia, stiffness and weakness. It was felt that skeletal muscle was the major source of elevated serum enzymes in patients undergoing therapy with clofibrate. A high incidence of muscular symptoms associated with elevated enzymes was reported in a study of patients with the nephrotic syndrome and hyperlipidaemia treated with clofibrate (25). The authors proposed that the higher percentage of unbound plasma CPIB secondary to the hypoproteinaemia was responsible for the toxic side effects of the drug. However one of their symptomatic patients had normal serum albumin and normal serum CPIB levels indicating that a high level of unbound plasma CPIB is not the only factor involved in the toxicity of this agent. Patient 4 in our study was the only one who developed elevated CPK levels during clofibrate therapy. He had normal renal function and normal plasma proteins. His fasting plasma CPIB concentrations was 86 µgm/ml, CPIB half-life was 9.6 hours and the drug pool size was 1608 mg. None

of these parameters were strikingly different from those in patients with normal plasma CPK values.

A recent report has indicated the possibility of gall stone formation in patients on long-term clofibrate therapy (12). Analysis of bile obtained via a catheter in the common bile duct of 7 post-cholecystectomised patients showed that clofibrate administration resulted in a striking decrease in the concentration of bile salts and an increase in the concentration of cholesterol, resulting in more lithogenic bile being secreted.

An effect of clofibrate therapy which is of clinical importance is the reduction in the requirement of anti-coagulants (26). The mechanism of anticoagulant potentiation of clofibrate is probably based on its ability to competitively displace albumin bound anticoagulant drugs, resulting in an increase in the active unbound anti-coagulant fraction. Bleeding may complicate matters unless this property of clofibrate is kept in mind. Clofibrate given to patients not on anticoagulants has no effect on the prothrombin time (9).

There have been conflicting reports in the literature regarding the effects of clofibrate on coagulation, in particular fibrinolysis (27,28,29). There appears to be general agreement however that the drug reduces plasma fibrinogen levels (27,28). Decreased platelet stickiness was demonstrated in patients with ischaemic heart disease given Atromid for a month (30). The report of Krasno

and Kidera (31) indicating that clofibrate administered to hyperlipidaemic patients resulted in a lowering of the incidence of nonfatal myocardial infarction whether or not plasma lipid levels were reduced is of interest and may reflect such anti-platelet clumping effect of the drug. However, decreased platelet stickiness was found to be temporary in another study utilising clofibrate over a longer period of time (29).

An interesting property of clofibrate, discovered recently, is its anti diuretic hormone like action. This is believed to be due to the release of endogenous anti diuretic hormone by the drug (32).

The administration of clofibrate in man results in the reduction of the triglyceride rich Sf 20-400 very low density lipoproteins and, to a lesser extent, the cholesterol rich Sf 0-20 low density lipoproteins (33). In a study of 33 patients, 31 had a decrease in the Sf 20-400 fraction whereas only 18 of 33 showed a decrease in the Sf 0-20 fraction. As would be expected this results in a greater depression of serum triglycerides than of serum cholesterol (22,34).

It has been shown that clofibrate reduces the level of plasma free fatty acids in man (23) and in expermental animals (35) and it has been postulated that a reduction of circulating levels of free fatty acids may result in a decreased availability of acetate for hepatic lipid synthesis. That this was not the whole

explanation, however, was indicated by the demonstration in the rat that both the in vivo and in vitro acetate incorporation into liver cholesterol was markedly inhibited by clofibrate. Incorporation of labelled mevalonate however was unimpaired, indicating that the drug decreases the rate of formation of mevalonate from acetate (36).

In man, interruption of the cholesterol biosynthetic pathway after the desmosterol stage by clofibrate was shown to be unlikely because desmosterol and related sterols were not detected in 11 patients at the time of the maximal hypocholesterolaemic response to clofibrate (37). In a recent study (38) it has been shown that clofibrate increases the clearance of triglycerides in extrasplanchnic tissues in patients with endogenous hyperlipidaemia.

Grundy, Ahrens and colleagues (39) have studied the mechanism of action of clofibrate in hyperlipidaemic patients using sterol balance and isotope kinetic techniques. They showed that clofibrate therapy resulted in an increase in the faecal excretion of neutral steroids and a decrease in faecal bile acid excretion. Total faecal steroid excretion, however, increased. Examination of the bile in some of their patients showed an increase in cholesterol and a decrease in bile salt excretion. Plasma cholesterol specific activity decay curves tended to flatten when clofibrate was administered.

Estimation of the size of cholesterol pools by isotope kinetic means showed decrease in total body cholesterol pools in 4 of 5 patients. These results indicate that clofibrate therapy results in increased excretion of sterols, most likely derived from cholesterol stored in tissues, in the face of decreased synthesis. In support of this conclusion is the observation that the size of xanthomas in skin and tendons can be reduced by clofibrate (40) and that the drug may lead to improvement in retinal exudates in patients with diabetic retinopathy (41).

Inspite of these extensive investigations, however, it must be stressed that at the present time the exact mechanism of action of clofibrate is not fully understood and probably involves multiple factors.

Some patients, particularly those with Type II hyperlipidaemia do not respond satisfactorily to clofibrate alone. In an effort to overcome this, combined drug therapy has been undertaken. In a study of 12 patients with coronary artery disease associated with serum lipid abnormalities (42) it was shown that clofibrate alone and d-thyroxine alone resulted in a sustained reduction in serum cholesterol. The combination, however, was found to be more effective than either drug used alone.

Neomycin, a nonabsorbable antibiotic, reduces plasma cholesterol levels when given by mouth (43,44,45). In

a study carried out by Samuel and his colleagues (46) it was shown that the combination of neomycin and clofibrate was more effective in reducing cholesterol levels in 4 of 11 patients with Type II hyperlipidaemia than administering either one of these drugs alone. In another report (47) the addition of neomycin to clofibrate therapy decreased plasma cholesterol concentration by an additional 12% in 42 hyperlipidaemic subjects (35 Type II and 7 Type IV).

The exact mode of action of oral neomycin in decreasing plasma cholesterol levels is unclear. Sedaghat et al. (48) have shown that the drug decreases cholesterol absorption. Large doses of oral neomycin (12 gm/day) results in a syndrome resembling idiopathic steatorrhoea (49). In view of the possibility that neomycin may interfere with clofibrate absorption we measured fasting plasma CPIB levels in patient 2 during clofibrate therapy alone and during the combined administration of neomycin and clofibrate. The difference was not statistically significant indicating that oral neomycin given in doses of 1 gm twice daily probably does not interfere with clofibrate absorption.

In 1960 cholestyramine was shown to decrease plasma cholesterol levels in experimental animals (50). This drug is a quarternary ammonium anion exchange resin, with basic groups attached to a styrene-divinyl-benzene copolymer by carbon to carbon bonds (50). It has a

molecular weight greater than a million and is neither digested nor absorbed from the gastrointestinal tract. In experimental animals the drug binds bile salts and interferes with their absorption (51). This results in an increase in the faecal excretion of bile acids and to some extent neutral steroids as well (50). The effectiveness of cholestyramine in reducing plasma cholesterol levels in patients with certain types of hyperlipidaemia, particularly Type II hyperlipidaemia, has been amply confirmed (52,53). Plasma triglyceride levels, however, may be elevated by this agent (52). Studies by Moutafis and Myant (54) and Grundy, Ahrens and Salen (52) have shown that cholestyramine therapy in Type II hypercholesterolaemic patients results in increased faecal bile acid and neutral steroid excretion. Isotope kinetic data however indicate that cholesterol synthesis during cholestyramine therapy is increased (52,54), and the increase may equal the increased faecal loss, the net result being little or no change in the total mass of exchangeable cholesterol (54).

Clofibrate promotes the excretion of faecal neutral steroids whereas cholestyramine mainly increases faecal bile acid excretion. Clofibrate decreases cholesterol synthesis and if it can be shown to inhibit the increased cholesterol synthesis produced by cholestyramine, the combination may be useful in the treatment of patients with hypercholesterolaemia resistant to either drug

separately. It has been shown in the rat that clofibrate decreases the cholestyramine induced increase in the rate of hepatic cholesterol synthesis (55).

In view of the report that cholestyramine binding interferes with the enterohepatic circulation of digitoxin in man (56), the report by Danhof (57) of impaired absorption of I^{131} labelled oleic acid by large doses of cholestyramine and the report by Gallo and his associates of the delay caused by cholestyramine in the absorption of drugs such as phenobarbital, phenylbutazone and Warfarin in the rat (51), we felt it was important to determine whether cholestyramine interfered with the absorption of clofibrate given concomitantly.

Our studies showed that the administration of cholestyramine had no significant effects on fasting plasma CPIB levels, 24 hour urinary and faecal excretion of CPIB and the half-life and pool size of the drug. The only consistent finding was that the peak plasma CPIB level was delayed by concomitant cholestyramine therapy. In this way the absorption of clofibrate behaves similarly to the absorption of phenobarbital and phenylbutazone in being delayed by cholestyramine.

Reversible drug-protein binding is of considerable importance in drug storage and drug-receptor combination. In plasma, only that portion of the drug which is free and unbound to protein is diffusible and pharmacologically active. The protein most frequently involved in drug binding is

albumin. Acidic and basic drugs tend to combine mainly by electro-static or ionic binding to oppositely charged sites on the protein molecule (58). It is only that portion of the drug which is not bound to plasma proteins that is available for glomerular filtration and therefore the glomerular filtration of a drug is inversely related to the degree of protein binding. Equilibrium dialysis experiments indicate that 96% of plasma CPIB is albumin bound (13). We confirmed this by electrophoresing plasma containing labelled CPIB; 93% of the labelled material migrated with the albumin fraction.

Monkeys infused intravenously with 20 mg CPIB per kg body weight had undetectable levels of the drug in bile (13). In contrast dogs fed clofibrate excrete CPIB in the bile both as the free acid and the glucuronide conjugate (11). Analysis of bile from 5 patients on clofibrate in this study showed the presence of substantial amounts of CPIB. Conjugated CPIB accounted for 40% of the total and there was a significant correlation between plasma and bile levels of CPIB.

Although our findings indicate the presence of substantial amounts of CPIB in human bile (in terms of µg of the drug per ml), biliary CPIB is only a minor fraction of the total amount excreted by the body.

The biliary excretion of a substance depends upon its molecular weight and polarity. Appreciable biliary excretion of a drug (greater than 10% of the administered

dose) in man occurs when the molecular weight of the drug is greater than 500. Compounds with molecular weight less than this are excreted mainly in the urine (59,60). The molecular weights of CPIB and its glucuronide conjugate, 215 and 409 respectively, are such that only small amounts would be expected to be excreted in bile. Our findings confirm this expectation.

The absorption studies in our patients indicate that the drug is almost completely absorbed. CPIB absorption calculated according to the 'plasma ratio method' is inaccurate and shows considerable variation between blood samples obtained at different times. This method is therefore not satisfactory for measuring CPIB absorption in man.

The mean 24 hour urinary excretion of CPIB in our patients was 1811 mg. A daily intake of 2 g clofibrate is equivalent to 1768 mg of CPIB. Therefore it seems that the urine is the major route of excretion of the drug. This was supported by the finding that in two patients given labelled CPIB orally, 100% and 99% of the labelled material was recovered in the urine within 6 days. The range of CPIB excreted daily in the urine (1437 - 2240 mg) indicates that although each patient takes in 2 g clofibrate daily (1768 mg CPIB), the daily urinary excretion of the drug is not exactly that amount but sometimes less and at other times more than this.

The majority of drugs are metabolised in the body and transformed into other substances. This transformation may be spontaneous or, more commonly, enzyme induced. The metabolism of foreign compounds usually occurs in two phases. In the initial phase the compounds are oxidised, reduced or hydrolysed and in the second phase these compounds (which now have suitable groups such as hydroxyl, carboxyl and amino groups) are conjugated to produce more polar compounds which can be readily excreted. For example benzene a lipid soluble neutral foreign compound is first oxidised to phenol (a weak acid possessing a hydroxyl group) which is then conjugated with glucuronic acid to form phenyl glucuronide. This is excreted by the kidney.

Conjugation usually results in a decrease or loss of toxicity of a drug or foreign compound. The conjugated product consists of the foreign compound attached to a molecule made by the body. These conjugation reactions use adenosine triphosphate as a source of energy. The foreign compound or the conjugating substance is attached to an activated nucleotide and then transferred (by a transferring enzyme) to the conjugating agent or the foreign compound, as the case may be. These reactions occur mainly in the liver and the kidney. Conjugation with glucuronic acid is catalysed by the enzyme glucuronyl transferase. This process results in the transfer of glucuronic acid from uridine diphosphate glucuronic acid to the foreign substance to form the glucuronide (61).

It has been reported that 92-98% of CPIB excreted in the urine is in the form of the glucuronide conjugate (13). We found that in 7 patients the conjugate accounts for a mean of 61% (range 43-73%) of the total CPIB excreted in the urine. Although the pH of the urine samples analysed for CPIB was not measured, it is possible that acid or alkaline urine may have caused spontaneous hydrolysis of the CPIB conjugate, resulting in the wide variation of the proportion of the conjugate found in the different urine samples. However, in a recent study by Houin and co-workers (62) it was shown that in 4 of 5 patients given a single oral dose of 500 mg clofibrate, 32.4 ± 14.4% of the urinary CPIB was in the free form, the rest being conjugated. The findings of the above group are thus similar to those in this report.

Houin and his associates confirmed that clofibrate was almost entirely excreted in the urine. CPIB was the main metabolite and was eliminated both unchanged and after conjugation. The presence of a glucuronide derivative (Fig.9) was proved by the increased concentration of CPIB found after hydrolysis by β-glucuronidase. These workers also showed that an unknown conjugate of CPIB which could be hydrolysed by acid but which was not a sulphate derivative was also present in the urine.

What could this unknown conjugate or conjugates be? The major conjugation processes occurring in man other than glucuronic acid conjugation include glycine

conjugation, glutamine conjugation, sulphate conjugation, methylation, acetylation and cyanide detoxication (61). With regard to the conjugation of CPIB, other than its conversion to the glucuronide, the conjugating mechanisms that should be considered are glycine conjugation and glutamine conjugation. This is because the chemical group necessary for conjugation with these compounds is the carboxyl (COOH) group which CPIB possesses.

Glycine conjugation can take place in the liver as well as the kidney. Thus it is possible that all or a part of the unknown conjugate of CPIB excreted in the urine may be its glycine conjugate.

Glutamine conjugation is a reaction of arylacetic acids in man. Arylacetyl co enzyme A reacts enzymatically with L glutamine to form the conjugate. The glutamine conjugate of CPIB has not yet been detected and although it is possible that some of the unknown urinary conjugate of CPIB may be of this nature, it is unlikely because this reaction seems to be confined to arylacetic acids (61).

The excretion of drugs by the kidney may involve one or more of the following mechanisms:

1. Excretion of the drug in the glomerular fluid.

   Glomerular fluid is blood filtered in the glomeruli and is equivalent to the plasma minus its proteins and other substances of molecular weight greater than 40,000. The excretion of

a drug in this manner is purely passive. However, the subsequent fate of the filtered drug depends on its physico-chemical properties; polar compounds or ions are not lipid soluble and therefore do not diffuse back into the peritubular blood whereas non-polar compounds readily do so.

P-chlorophenoxyisobutyric acid is mainly albumin bound in the plasma. Thus only that fraction which is not protein bound (approximately 7%) is available for filtration at the glomeruli. This compound being non-polar would be expected to diffuse back through the renal tubular cells into the peritubular blood. Thus glomerular filtration and excretion of the drug by this mechanism probably plays only a minor role in the overall excretion of the drug.

2. Proximal tubular secretion.

This is an important route of excretion for many foreign substances. The process is an 'active' one which means that energy is required for it to take place. Both organic acids and organic bases may be actively secreted by the proximal tubules. Examples include hippuric acid, aromatic sulphates and glucuronides, phenol red, diodone and some

of the penicillins. In contrast to drug excretion in glomerular fluid, in tubular secretion of drugs, both the bound and unbound drug are available. As unbound drug is secreted, equilibrium is shifted so as to allow rapid dissociation of the drug-albumin complex. The degree of availability of the bound drug, however, depends on the drug's affinity for the active transport mechanism (63).

The urinary excretion of CPIB may well involve this mechanism of 'tubular secretion'. Active tubular secretion of a substance may be suspected when the clearance of that substance is greater than the glomerular filtration rate (64). Although data regarding the creatinine clearance (= glomerular filtration rate) was available in patients 2-6, it was not possible to determine the renal plasma clearance of CPIB accurately. This was because P (the plasma concentration in mg per ml of CPIB) in the formula $\frac{UV}{P}$ (needed to calculate the renal plasma clearance of the drug, where U = the urinary concentration of CPIB in mg per ml and V = urine volume in ml per minute) was not constant. The patients under study were taking 1 g clofibrate twice

daily by mouth. This resulted in basal plasma CPIB levels before drug intake and peak levels several hours after taking the drug i.e. the plasma CPIB concentration was continuously changing, making it impossible to measure the renal plasma clearance of CPIB with any degree of accuracy.

The best way to determine renal plasma clearance of CPIB would have been to infuse the drug intravenously in such a way as to achieve a steady known plasma level of the drug. The clearance could then have been calculated by measuring the urine volume and the urine concentration of CPIB during the steady state of the infusion.

If the plasma clearance of CPIB measured in such a way was found to be greater than the glomerular filtration rate as determined by the creatinine clearance, then renal tubular excretion of CPIB could be presumed. On the other hand, if found to be lower, renal tubular excretion could not be excluded. This is because although renal plasma clearance of CPIB measured in the above manner may be less than the creatinine clearance, the clearance of CPIB measured using the unbound fraction of plasma CPIB (that fraction of plasma CPIB which is not

protein bound and thus available for glomerular filtration) may be higher than the creatinine clearance.

3. Tubular diffusion.

Drugs may be excreted into the urine by a process termed non-ionic diffusion. This process was first described by Milne and his associates in 1958 (65). The process is 'passive' in that energy is not required for it to take place. The properties that a weak acidic or basic compound must have before excretion by diffusion can occur include a rapid diffusion of the unionised component across the cell membrane, a relative impermeability of the tubular cells to the ionised fraction and a dissociation constant (pK_a) within the range of 3.0-7.5 for weak acids and 7.5-10.5 for weak bases.

Proof of non-ionic diffusion rests on the demonstration that a change in urinary pH has a profound effect on the renal clearance of the compound. It is important to note that in excretion by non-ionic diffusion clearance is influenced only by the urinary pH and not by effects on total body acid-base balance. Examples of renal excretion by non-ionic diffusion of weak acids include

salicylic acid (66), phenobarbital, nalidixic acid and nitrofurantoin, and of weak bases that of chloroquine, mecamylamine (67), pethidine (68), nicotine, procaine and quinine.

The discovery of the principle of non-ionic diffusion was of the utmost importance in the management of cases of poisoning. Thus making the urine alkaline promotes the excretion of salicylic acid and phenobarbitone in cases of poisoning with these compounds and making the urine acidic promotes the excretion of amphetamine and pethidine in cases of overdose with these agents.

With regard to the excretion of free CPIB, this compound is non-polar and would therefore be expected to diffuse rapidly across tubular cell membranes. On the other hand, ionised CPIB is polar and would probably not diffuse too readily across the renal tubular cells. In view of these properties and the fact that the pK_a of CPIB is 3.0 (13) it seems very likely that the drug is excreted into the urine by non-ionic diffusion. If this were the case then it would be interesting to hypothesize that the variability in the 24 hour urinary CPIB excretion between the different patients might be explained on the basis of differing urinary pH values; patients with alkaline urine excreting more than those with acidic urines. Similarly the variability in the proportion of free and conjugated urinary CPIB could be explained by the above hypothesis; more free CPIB would be excreted in alkaline urine and less in acid urine.

Measurements of CPIB pool size showed no patients with drug pool size greater than the daily CPIB intake. Pool size was dependent on the interval between the last dose of clofibrate and the time measurements were made; the mean pool size in 3 patients measured 3 hours after the morning dose of clofibrate was 1560 mg. Two patients who were not given their morning dose of the drug (the interval between their last dose of clofibrate and drug pool size measurement being 18 hours) had a mean pool size of 730 mg. There was no positive correlation between duration of clofibrate therapy and drug pool size, indicating that drug accumulation in patients on long term treatment does not occur. The mean half-life of plasma CPIB in 5 patients was 15 hours. This correlates well with the finding by Thorp of a half-life of 14 hours in 4 normal subjects calculated from non isotope kinetic data (13).

Of the many factors which affect drug metabolism such as the animal species and strain being investigated, age, chronic administration, route of administration and the concomitant administration of other drugs, the latter is of particular importance in the present study. It is a well known fact that the pharmacokinetics of drugs can be influenced by the administration of other drugs given simultaneously. For example, phenobarbitone stimulates enzyme activity in the liver cell endoplasmic reticulum resulting in the more rapid hydroxylation of hexobarbitone

and the demethylation of meperidine (69). Diphenylhydantoin is another example of such "enzyme inducing" compounds. Thus when the metabolism of a certain drug is being studied it is imperative to know whether other drugs are being taken at the same time. None of our patients were on drugs other than clofibrate except those given cholestyramine and neomycin over short periods of time.

The delay caused by cholestyramine in the absorption of CPIB should affect biliary CPIB as well as that given by mouth. Half-life of the drug would be expected to be prolonged. This did not occur in the 3 patients studied. The explanation appears to be that the amount of CPIB arriving in the gut via the bile, when absorbed, is only a very small fraction of the total pool and that delayed reabsorption of this fraction has little effect on CPIB half-life. Assuming a 24 hour biliary flow rate of 1000 ml and a 24 hour biliary CPIB concentration of 100 µgm/ml (mean fasting biliary CPIB concentration was 55 µgm/ml in 5 patients), the total amount of CPIB absorbed from bile in 24 hours would be 100 mg. This is 5.6% of the total CPIB absorbed from the gut during that time.

Although the liver can conjugate CPIB, the amount of the conjugate absorbed from the bile (assuming a biliary flow rate of 1000 ml per day, a 24 hour biliary CPIB concentration of 100 µgm/ml 50% of which is conjugated) would be approximately 50 mg daily. This

would account for only 5% of the urinary conjugate excreted in 24 hours. No conjugate of CPIB is detectable in the plasma; the absorption of 50 mg of the conjugate from the bile over a period of 24 hours would result in undetectable plasma levels of the compound. These findings point to the kidneys as the main site of conjugation. The CPIB conjugate in the urine of clofibrate fed dogs has been shown to be the glucuronide by mass spectroscopy (11). Thorp (personal communication) has shown that rat kidney slices incubated with free CPIB produce the glucuronide and the recent report by Houin et al. confirms the presence of CPIB glucuronide in the urine of patients given clofibrate by mouth (62). In man the kidney has long been known as a site of glucuronide formation (70). This organ contains uridine diphosphate glucuronic acid, and microsomes in the cortical tissue possess an enzyme similar to liver uridine diphosphate transglucuronylase which has the ability to transfer glucuronic acid from the nucleotide to acceptors (71).

It is believed that in monkeys CPIB is distributed in the extracellular fluid compartment of the body. No drug was detectable in muscle, heart or spleen of animals infused intravenously with 20 mg per kg body weight of the sodium salt of CPIB (13). In an attempt to determine whether clofibrate was present in other body tissues in man we carried out adipose tissue biopsies in 4 patients. In three no drug was detectable. In spite of this finding,

our calculations in 5 patients (Table 12) show that the total amount of CPIB in plasma is much smaller than the drug pool size calculated by isotope kinetic data. Although we have not had the opportunity of measuring CPIB in interstitial fluid it is probable that CPIB is bound to albumin which is present in that compartment. Giltin and colleagues (72) have calculated that in man the quantity of albumin in the interstitial fluid is approximately equal to that in the vascular compartment. Assuming that CPIB is bound to albumin in the interstitial fluid to the same extent as plasma albumin, the total extracellular fluid CPIB, that is the total plasma and interstitial fluid CPIB content, continues to be appreciably smaller than the total pool size based on isotope kinetic data. These findings indicate that the drug is present in other tissues of the body. The presence of CPIB in bile indicates that the drug is probably present in the liver cells and for the kidneys to conjugate CPIB the drug must enter renal parenchymal tissue.

In summary, cholestyramine or neomycin may be administered along with clofibrate without fear of any interference with its absorption. Clofibrate given orally is almost completely absorbed, it is present in the plasma entirely in the form of CPIB, over 90% of which is albumin bound. There is no detectable CPIB conjugate in the plasma. The kidney is probably the main site of conjugation of the drug and the urine is

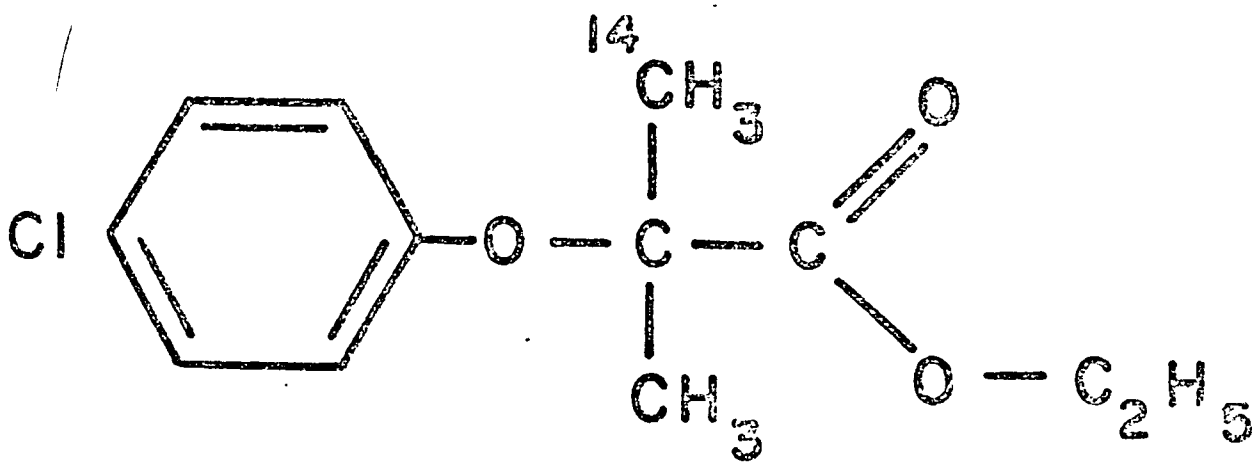
the major route of its excretion. In vivo degradation of CPIB does not occur. CPIB is present in the bile and is probably distributed in tissues other than the extracellular fluid compartment of the body.

Table 1

CLINICAL DATA

PATIENT	INITIALS	AGE	SEX	WEIGHT Kgm	DIAGNOSIS
1	A.B.	24	F	65	Hypercholesterolaemia
2	A.J.	72	M	61	Normolipidaemia, C.O.P.D.*
3	D.R.	63	F	56	Endogenous hyperglyceridaemia
4	J.A.	54	M	89	Hypercholesterolaemia
5	R.H.	44	M	101	Broad beta disease
6	M.B.	68	F	59	Hypercholesterolaemia, hypertension
7	A.C.	17	F	34	Hypercholesterolaemia, post ileal resection
8	M.R.	30	F	56	Hypercholesterolaemia
9	J.G.	57	M	63	Hypercholesterolaemia
10	T.N.	40	M	91	Hypercholesterolaemia
11	J.T.	43	F	52	Cholelithiasis
12	D.C.	34	F	65	Cholelithiasis
13	F.A.	34	F	56	Cholelithiasis
14	L.B.	51	F	74	Hypercholesterolaemia, cholelithiasis
15	A.R.	33	F	54	Cholelithiasis

* C.O.P.D. = chronic obstructive pulmonary disease.



^{14}C - Labelled Clofibrate

Figure 1

Table 2

PLASMA CPIB ^{3}H TO ^{14}C RATIO IN PATIENT 4 INFUSED INTRAVENOUSLY WITH A MIXTURE OF THE ISOTOPES

TIME* (hrs)	PLASMA CPIB $^{3}H/^{14}C$ RATIO**
0.5	1.73
1	1.70
2	1.60
3	1.62
4	1.58
5	1.64
6	1.66
20	1.77

* Following intravenous infusion.

** $^{3}H/^{14}C$ ratio infused intravenously was 1.64.

Table 3

FASTING PLASMA CPIB CONCENTRATION

PATIENT	CLOFIBRATE DOSE (mg/kgm body wt./day)	DURATION OF THERAPY (weeks)	FASTING PLASMA CPIB* (µgm/ml ± S.D.)
1	31	7	90 ± 31 (n = 3)
2	33	3	156 ± 6 (n = 3)
3	36	416	114 ± 31 (n = 3)
4	23	104	86 ± 15 (n = 4)
5	20	208	82
6	34	156	123
8	36	3	123 ± 12 (n = 3)
11	38	2	159 ± 20 (n = 4)
12	31	2	165
13	36	2	109
14	27	2	114
15	37	2	157

* Plasma CPIB concentration before the morning dose of clofibrate.

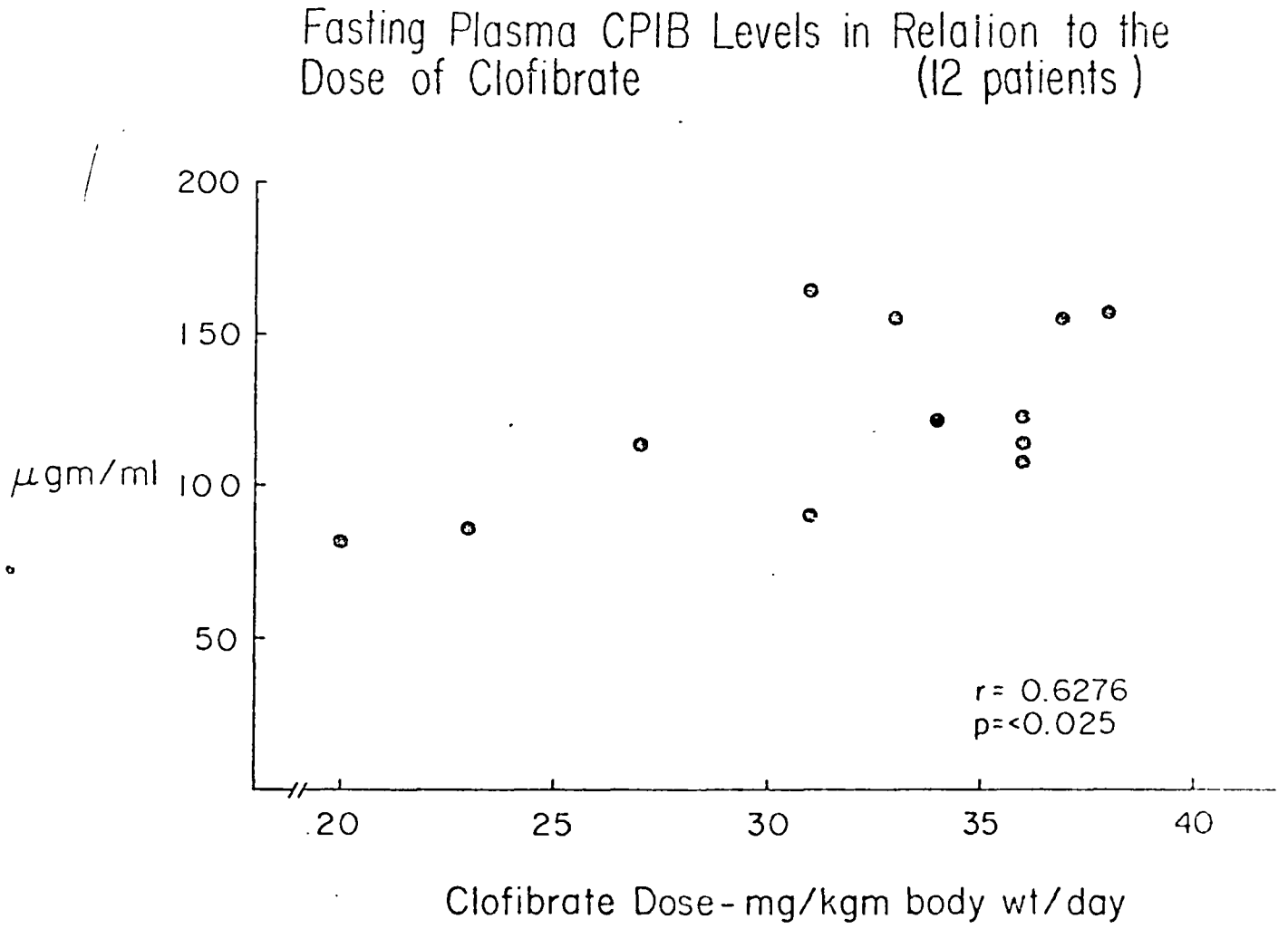

Fasting Plasma CPIB Levels in Relation to the Dose of Clofibrate (12 patients)
200
150
100
50
μgm/ml
r = 0.6276
p=<0.025
20
25
30
35
40
Clofibrate Dose - mg/kgm body wt/day

Figure 2

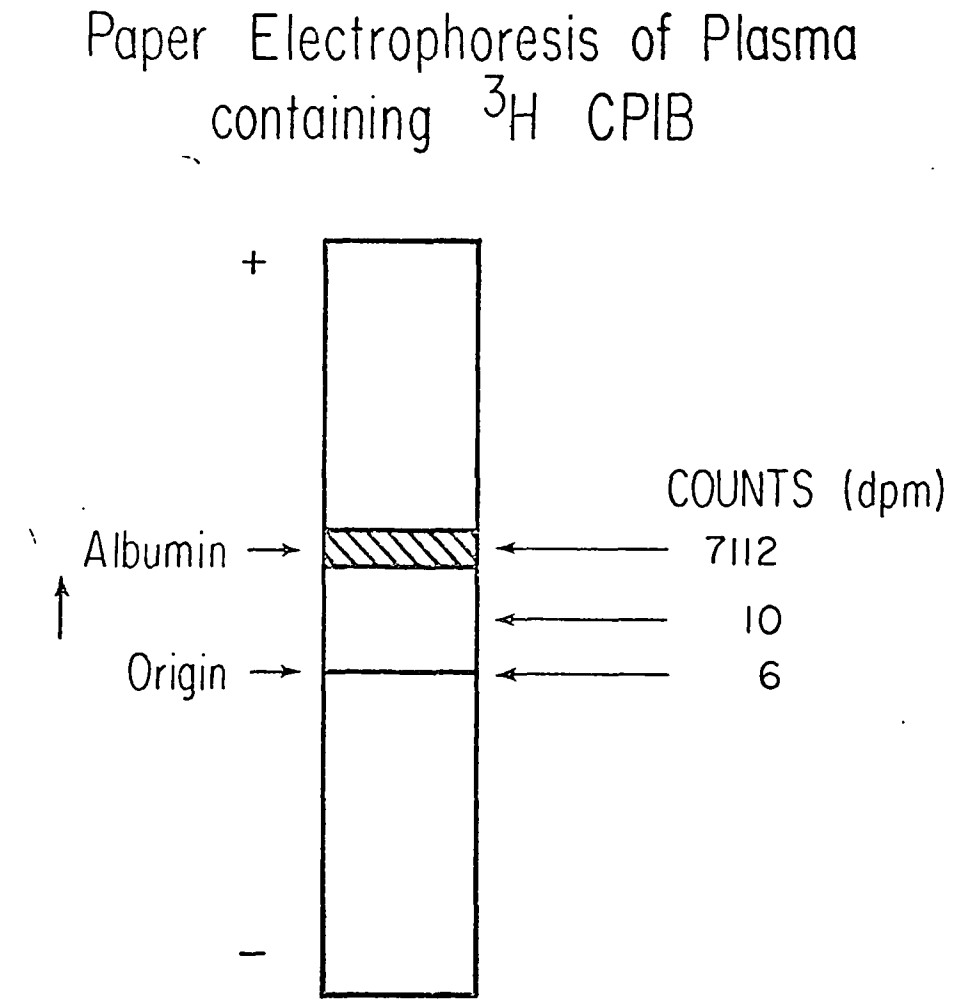


Figure 3. 200 µl of plasma containing 7,665 dpm of ^{3}H-CPIB was electrophoresed. 93% of the radioactivity was recovered from the albumin band.

Table 4

FASTING BILIARY CPIB CONCENTRATION

PATIENT	BILIARY CPIB* (µgm/ml ± S.D.)	CONJUGATED CPIB (%)	PLASMA CPIB (µgm/ml ± S.D.)
11	48 ± 19 (n = 6)	11	157 ± 20 (n = 4)
12	81	40	165
13	20		109
14	36		114
15	89	70	157

* Measured before the morning dose of clofibrate.

Table 5

DATA ON CPIB ABSORPTION

PATIENT	ABSORPTION BY METHOD I (%)	ABSORPTION BY METHOD II (% ± S.D.)
2	99.83	76 ± 5 (n = 8)
3		90 ± 27 (n = 8)
5	99.98	116 ± 29 (n = 10)
6	99.00	117 ± 12 (n = 11)

Table 6

FAECAL EXCRETION OF CPIB

PATIENT	CLOFIBRATE DOSE* (mg/day)	FAECAL EXCRETION OF CPIB (mg/day ± S.D.)	(% of daily dose)
2	2000	39 ± 45 (n = 2)	2
7	1500	140 ± 58 (n = 2)	10
8	2000	81 ± 14 (n = 2)	5

* Each 500 mg capsule of clofibrate contains 442 mg CPIB.

Table 7

EXCRETION OF CPIB IN THE URINE

PATIENT	URINE CPIB (mg/day ± S.D.)	(% of daily dose)*	CONJUGATED CPIB IN URINE (%)
1	1437 ± 494 (n = 2)	81	71
2	1495 ± 88 (n = 4)	85	50
3	1943 ± 279 (n = 2)	110	58
4	2057	116	73
5	1305	74	61
9	2200	124	69
10	2240	127	43

* All patients received 2 g clofibrate daily (equivalent to 1768 mg CPIB).

Table 8

ANALYSIS OF ADIPOSE TISSUE FOR CPIB IN PATIENTS ON LONG-TERM CLOFIBRATE THERAPY

PATIENT	MASS OF ADIPOCYTES ANALYSED	CPIB CONTENT OF ADIPOCYTES	LABELLED CPIB CONTENT OF ADIPOCYTES*	PLASMA LABELLED CPIB CONTENT
	(mg)	(µgm)	(dpm)	(dpm/ml)
1	678	0		
2	204	0	0	4418
3	253	12	8	5685
4	308	0	29	2185

* Patients 2-4 were infused with 10-20 µci of ^{3}H CPIB 2-3 hours prior to adipose tissue biopsy.

Table 9

HALF-LIFE AND POOL SIZE OF CPIB IN PATIENTS ON LONG-TERM CLOFIBRATE THERAPY

PATIENT	CREATININE CLEARANCE*	CPIB KINETIC DATA		CPIB MASS MEASUREMENTS	
		t 1/2	pool size	t 1/2	pool size
	(ml/min)	(hrs)	(mg)	(hrs)	(mg)
2	37	17.0	1980	16.5	
3	97	5.7	1090		
4	124	9.6	1608		
5**	164	20.0	709		613
6**	79	24.3	751	24.0	847

* Normal creatinine clearance 110-150 ml/min.

** Patients 5 and 6 were not given their morning dose of clofibrate on the day of study.

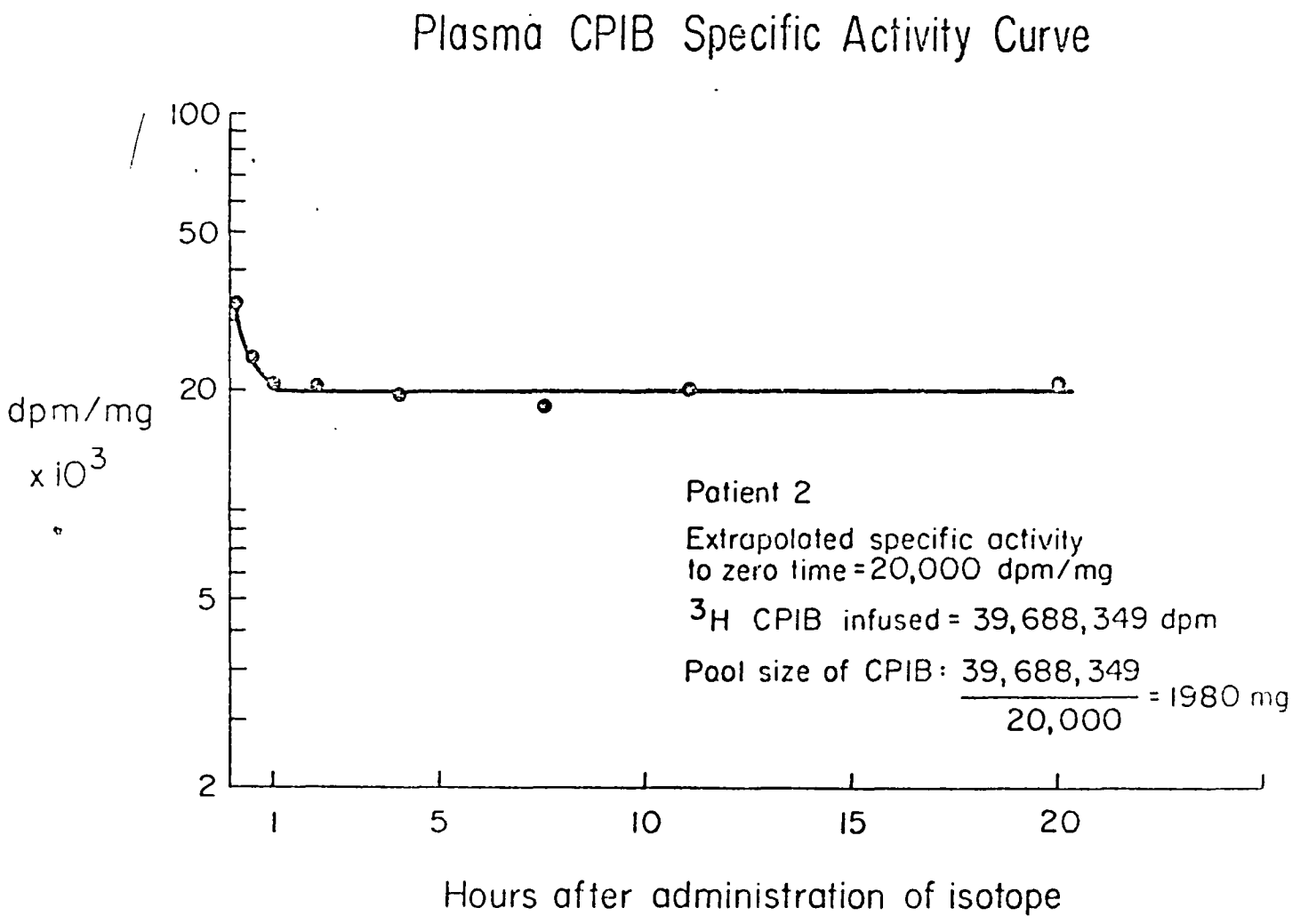

Plasma CPIB Specific Activity Curve
100
50
20
5
2
dpm/mg
x 10^3
Patient 2
Extrapolated specific activity
to zero time = 20,000 dpm/mg
3H CPIB infused = 39,688,349 dpm
Pool size of CPIB: 39,688,349 / 20,000 = 1980 mg
1
5
10
15
20
Hours after administration of isotope

Figure 4

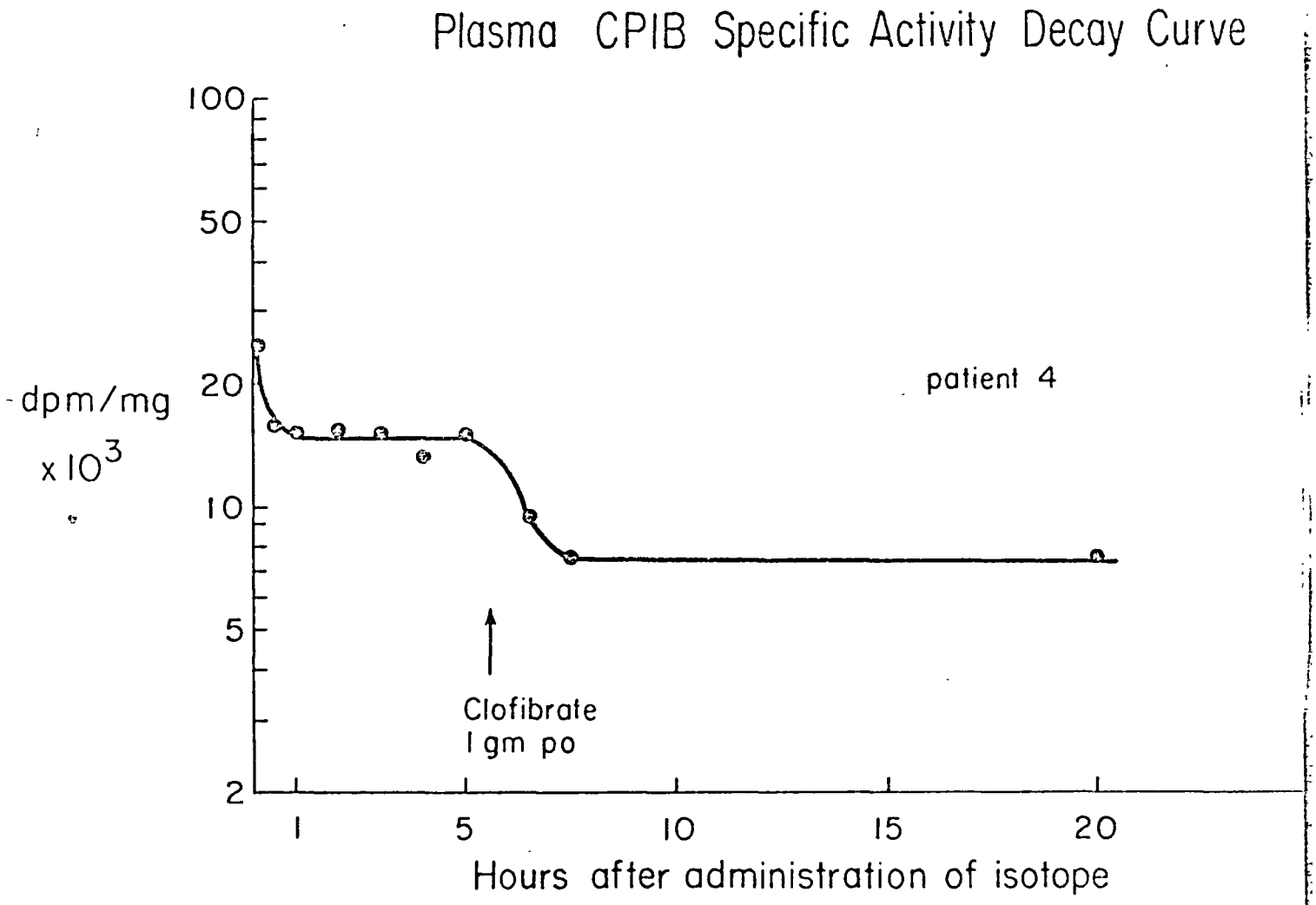

Plasma CPIB Specific Activity Decay Curve
dpm/mg
x 10^3
100
50
20
10
5
2
patient 4
Clofibrate
1 gm po
1
5
10
15
20
Hours after administration of isotope

Figure 5

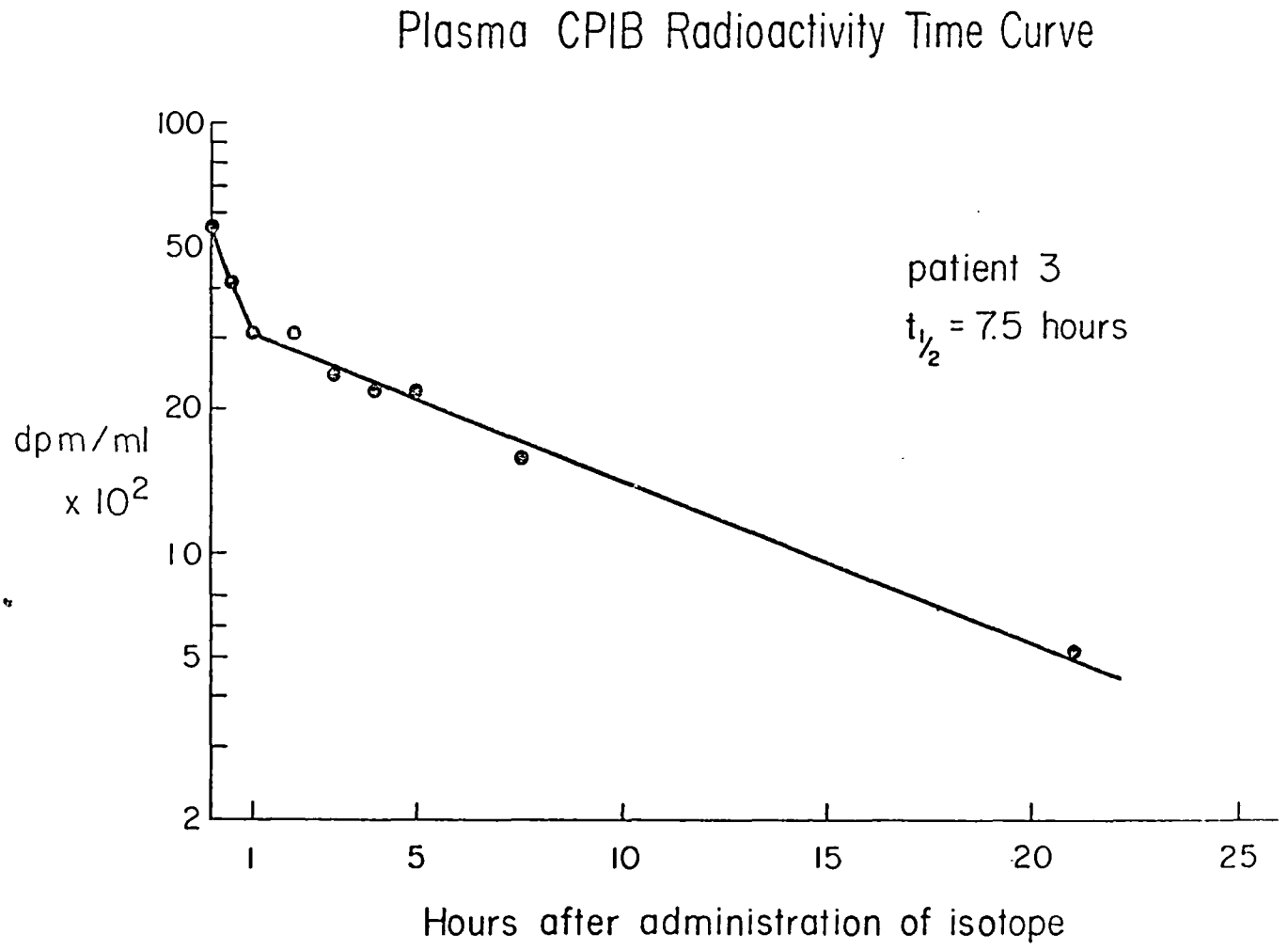

Plasma CPIB Radioactivity Time Curve
100
50
20
10
5
2
dpm/ml
x 10²
patient 3
t½ = 7.5 hours
1
5
10
15
20
25
Hours after administration of isotope

Figure 6

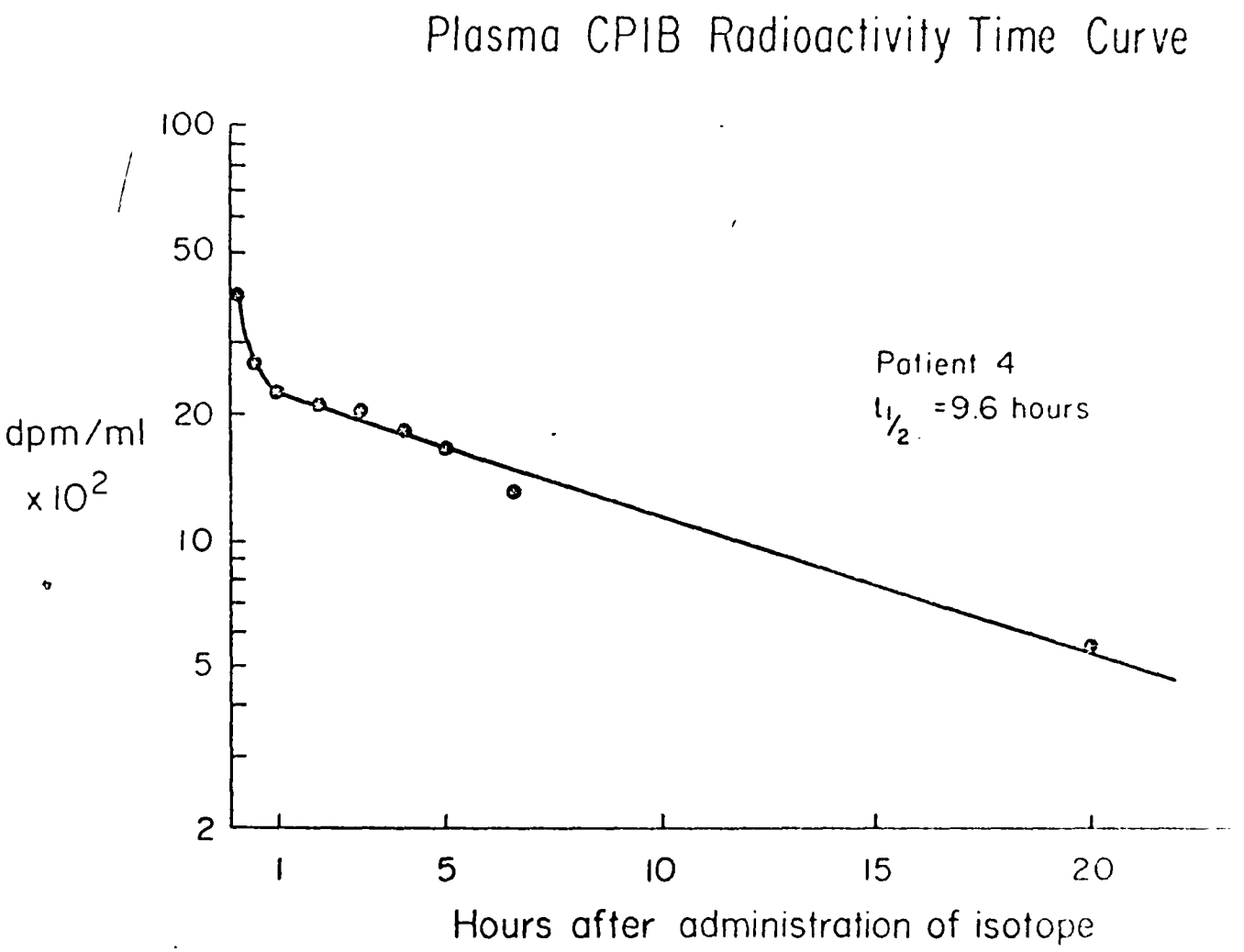

Plasma CPIB Radioactivity Time Curve
100
50
20
10
5
2
dpm/ml
x 10²
Patient 4
t1/2 =9.6 hours
1
5
10
15
20
Hours after administration of isotope

Figure 7

Table 10

CPIB METABOLISM DURING THE ADMINISTRATION OF CLOFIBRATE (I)

AND CLOFIBRATE PLUS CHOLESTYRAMINE (II)

PATIENT	FASTING PLASMA CPIB CONCENTRATION µgm/day ± S.D.(n)		URINARY EXCRETION OF CPIB mg/day ± S.D.(n)		FAECAL EXCRETION OF CPIB mg/day ± S.D.(n)	
	I	II	I	II	I	II
1	90 ± 31 (3)	84	1437 ± 494 (2)	1672		
2	156 ± 6 (3)	184 ± 21 (3)	1495 ± 88 (4)	1779	39 ± 45 (2)	62 ± 9 (2)
3	114 ± 31 (3)	106 ± 3 (2)	1943 ± 279 (2)	1913		
4	86 ± 15 (4)	82 ± 6 (2)	2057	1537		
7					140 ± 58 (2)	54 ± 2 (2)
8	123 ± 12 (3)	130 ± 15 (4)			81 ± 14 (2)	147 ± 166 (2)

Table 11

CPIB METABOLISM DURING THE ADMINISTRATION OF CLOFIBRATE (I) AND CLOFIBRATE PLUS CHOLESTYRAMINE (II)

PATIENT	CPIB t 1/2 (hrs)		CPIB POOL SIZE (mg)		TIME OF PEAK PLASMA CPIB* (hrs)	
	I	II	I	II	I	II
2	17.0	15.5	1980	1730	3.5	7.5
3	5.7	7.5	1090	1800	3.5	5.0
4	9.6	8.8	1608	1562	4.0	6.0

* Time taken to achieve peak plasma CPIB level following the intake of drug(s) with breakfast.

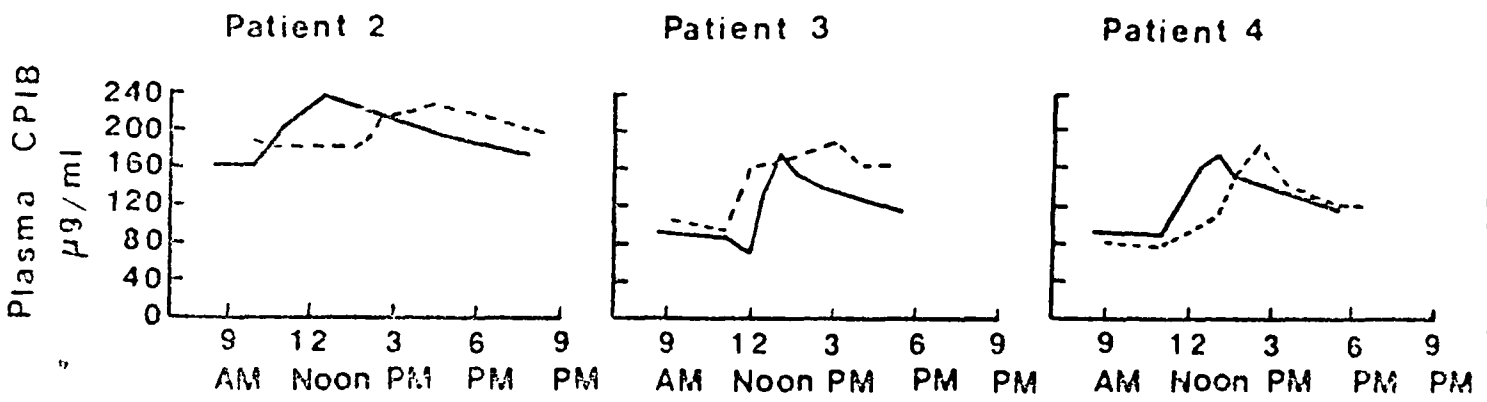
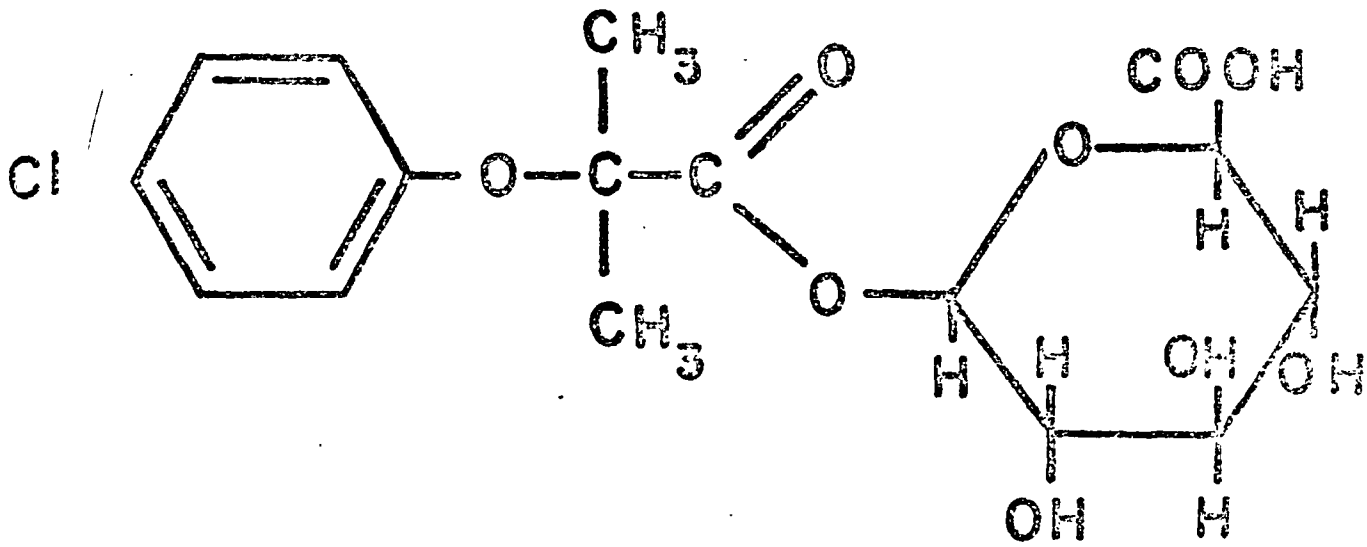


Figure 8. The effect of cholestyramine (4 g four times daily) on the time taken to achieve peak plasma CPIB levels in patients on clofibrate (1 g twice daily)

_____ clofibrate

---------- clofibrate + cholestyramine



The Glucuronide Conjugate of

p-Chlorophenoxyisobutyric Acid

Figure 9

Table 12

THE DISTRIBUTION OF CPIB IN THE BODY OF PATIENTS ON LONG-TERM CLOFIBRATE THERAPY

PATIENT	POOL SIZE OF CPIB[1] (mg)	TOTAL PLASMA CPIB CONTENT[2] (mg)	TOTAL PLASMA AND INTERSTITIAL FLUID CPIB CONTENT[3] (mg)	TOTAL POOL SIZE MINUS EXTRACELLULAR CONTENT[4] (mg)
2	1980	598	1196	784
3	1090	342	684	406
4	1608	564	1128	480
5	838	305	610	228
6	751	262	524	227

[1] Calculated from isotope kinetic data.

[2] Obtained by multiplying the plasma CPIB concentration by the plasma volume (73).

[3] The interstitial fluid CPIB content was assumed to be equal to the CPIB present in the intravascular compartment. This assumption is based on the fact that albumin which binds over 90% of CPIB is present in interstitial fluid in amounts equal to that in the plasma (72).

[4] CPIB not accounted for in plasma and interstitial fluid space is assumed to be distributed in tissues.

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ADDENDUM

Since the initial preparation of this thesis two articles based on the data presented in it have been published.

1. Sedaghat, A., Nakamura, H., and Ahrens, E.H., Jr. Determination of clofibrate in biological fluids by thin-layer and gas-liquid chromatography. J. Lipid Res. 15: 352-355, 1974.

2. Sedaghat, A., and Ahrens, E.H., Jr. Lack of effect of cholestyramine on the pharmacokinetics of clofibrate in man. Europ. J. Clin. Invest. 5: 177-185, 1975.