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Title: EFFECTS OF PENTACHLOROPHENOL ON CULTURES OF EMBRYONIC CELLS FROM ONCORHYNCHUS KISUTCH

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Embryonic tissue from coho salmon was grown in Eagle's minimum essential medium supplemented 20 % with newborn, agamma calf serum at 23°C. At the end of a ten day growth period control, 40 mg potassium pentachlorophenate (KPCP) per liter and 80 mg KPCP/l poisoned cultures were analyzed for cell numbers, packed cell volume, dry weight, ash residue weight, nitrogen and derived fatty acid methyl esters (FAME). Depleted media from these cultures were analyzed for dry weight, ash residue weight and derived FAME. Individual fatty acid methyl esters were identified and estimated quantitatively by gas-liquid chromatography and by weighing total FAME.

Growth inhibition was induced by KPCP poisoning and the percentage of inhibition was a linear funtion of KPCP concentration. Fifty percent inhibition occurred at 66 mg KPCP/l with seven days

exposure. Cell populations tolerated 80 mg KPCP/1 for 30 days (two cell passages) but could not survive 120 mg KPCP/1 for this time period. Control populations increased their cell numbers 2.4 times the inoculum value during the ten day growth period. The average cell in control populations had a volume of 1873×10^{-12} ml and contained 194 pg of dry matter, 23.4 pg of matter after ashing, 28.7 pg of nitrogen, 21.0 pg of derived fatty acid methyl esters and 1717 pg of water. The magnitude of all of these cell characteristics, with the exception of water, decreased with 40 mg KPCP/1, but did not reach a level with statistical significance. The magnitude of the characteristics increased with 80 mg KPCP/1 and the increases were statistically significant. The concentrations of dry matter, ash, nitrogen and fatty acids per ml of cells, upon poisoning with 40 mg KPCP/1, decreased to a statistically significant level. After poisoning with 80 mg KPCP/1 the material concentrations also decreased significantly with the exceptions of ash and nitrogen whose differences were not statistically significant and of FAME, whose concentration was significantly increased.

The percentage contributions of individual fatty acids to total fame differed between day zero medium, control populations and day ten depleted media indicating selective catabolism and anabolism. Control populations generally used up saturated acids,

removed monene acids from the medium and stored them in cells and synthesized branch chain and polyunsaturated fatty acids. Poisoning populations with 40 mg KPCP/l and 80 mg KPCP/l generally did not alter the selective handling of individual fatty acids of control populations, but there was an excess net catabolism of fatty acids due to 40 mg KPCP/l and this excess loss was related to the concentration of individual fatty acids. A total of 2.74 mg of fatty acids disappeared from control cultures over a ten day period and 3.65 and 2.31 mg disappeared from cultures exposed to 40 mg and 80 mg of KPCP per liter, respectively.

Effects of Pentachlorophenol on Cultures of Embryonic Cells from Oncorhynchus kisutch

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INTRODUCTION

Tissue culture has been used extensively as a tool in the investigation of toxicological problems, most importantly in screening and comparing numerous chemicals for toxicity. Among the advantages of tissue culture in toxicological studies are the large population of living units that can be handled, rapid growth, short generation time and chemically well defined nutrition. Disadvantages in the use of tissue culture for toxicological problems include limitation in the route of toxicant administration, the necessity of testing only general cell poisons rather than poisons which act primarily on specific tissues or extracellular integrating systems and the unknown equalities of cells *in vitro* to cells *in situ*.

Generally toxicity in cultured cells is studied by change in cell growth or cell morphology with poisoning. Cell growth has been estimated by cell population numbers, population volume or elaboration of cell protein (16, 17, 18, 19, 20, 21, 27, 30).

This study examines cells and their metabolism under control and poisoned conditions to define more thoroughly the chemical and bioenergetic results of life processes and the effects of pentachlorophenol poisoning on them. The use of *in vitro* cultured cells

in metabolic studies has the same advantages and disadvantages mentioned above, however, there are the additional advantages of a metabolism uncomplicated by varying muscle activity and by such integrating mechanisms as the nervous system or endocrine glands. Cultured cells presumably utilize energy primarily for growth, replication and the maintenance of a living state.

Pentachlorophenol is an important industrial toxicant as well as a standard for agents which uncouple phosphorylation from oxidative catabolism (37, 38, 39, 40, 41). It is encountered by fish in their natural environment because of its extensive use as a preservative by the lumber industry. (The concentration in the Willamette River has been found as high as one part per million.) For these reasons pentachlorophenol has been used at Oregon State University in metabolic studies of fish enzymes and fish at various stages of development (9, 10, 11, 20, 21, 22, 25). This study was initiated to supplement existing data and to compare results between pentachlorophenol poisoning of fish and fish cell cultures in vitro.

The necessarily small samples of living material that can be derived from tissue cultures did not permit useful direct caloric investigation of cell components. Techniques are available, however, for the investigation of minute quantities of fatty acids. Since fatty acids are important as energy storage molecules as well as for structural integrity and since fatty acid metabolism has been studied

in coho salmon under control and pentachlorophenol poisoned conditions, the fatty acid compositions of fresh medium, cell populations and depleted media were determined and fatty acid utilization calculated in this study (22, 34, 35, author's unpublished data).

Specific groups of experiments were run to determine a short term index of poisoning (Inhibitory dose) and a long term index of pentachlorophenol poisoning (Tolerated Toxic Concentration). Other experiments were run to determine cell growth, its cost in material and the effects of growth on the composition of cells and their medium as well as the changes in growth and the changes in the composition of cells and their medium induced by pentachlorophenol poisoning.

MATERIALS AND METHODS

All chemicals were C. P. grade unless otherwise stated. Solvents, including water, were glass-distilled from drum stock or house distilled water. All glassware used in contact with the cells was thoroughly rinsed in glass-distilled water.

Cell Line

The cells in this study were derived by Fryer, Yusha and Pilcher from minced, trypsinized, embryonic tissue from the coho salmon (Oncorhyncus kisutch Walbaum). The cell line was designated CSE 119 (16). CSE 119 is heteroploid and fibroblast-like and had been carried through 65 culture passages when these experiments were begun.

Cell Culture Medium

Eagle's minimum essential medium (13) supplemented 20% with newborn agamma calf serum (Hyland Laboratories, Los Angeles, Calif.) was used throughout. Penicillin at 100 units/ml and streptomycin at 100 μg/ml were included in the nutrient medium. The cells were incubated at 23°C on the flat surface of 32 oz prescription bottles inoculated with 40 ml of medium containing 900,000 cells/ml. The newborn calf serum supplement had been fractionated to remove

gamma globulins. Compositions of and reagents used for the balanced salt solution and the minimum essential medium are given in Table I.

Cell Culture Techniques

The cells were grown at 23° C on the flat surfaces of 32 oz prescription bottles. Bottles were closed with white rubber, non-toxic stoppers and the necks were covered with sterile aluminum foil. The cells were subcultured at ten day intervals. After the spent medium was decanted, cells were removed from the glass for subculturing with a trypsin solution (See Table I). The cells were concentrated from the trypsin solution by centrifugation at 1000 rpm (284 x g) for ten minutes in an International Centrifuge, size 2, model V. The trypsin solution was decanted and the cells were dispersed in ten ml of medium by passing repeatedly (six times) through the tip of a pipette. For mere maintenance of the culture, the concentration of the cells was estimated from the density of the cells after ten days growth, and, by proper dilution of the ten ml suspension, the new culture was established at two, three or four times the volume of culture of origin.

Table I. Cell Culture Solutions.

Earl's Balanced Salt Solution (BSS)

The terms 10 X and 1 X identify relative solution concentrations with 1 X being the working concentration.

BSS (10 X)

NaCl	68.0 g
KCl	4.0 g
$MgSO_4 \cdot 7\ H_2O$	2.0 g
$NaH_2PO_4 \cdot H_2O$	1.25g
H_2O	quantitiy sufficient to dissolve salts
Phenol red	100 ml of 0.2% aqueous solution
$CaCl_2 \cdot 2\ H_2O$	2.65 g Add last as a solution.

Make up to one liter and autoclave. Store at 4° C.

BSS (1 X)

(10 X) BSS	100 ml
Glucose	1 g

Make up to one liter, autoclave and store frozen.

Eagle's Minimum Essential Medium (MEM)

All components must be sterile.

Earl's (1 X) BSS	375 ml autocalved and stored frozen
11% $NaHCO_3$	5 ml autoclaved and stored frozen
(50 X) MEM essential amino acids[a]	8 ml
(100 X) MEM non-essential amino acids [a]	4 ml
(100 X) Sodium pyruvate [a]	4 ml
(100 X) MEM vitamin mixture [a]	4 ml
	400 ml

(a) Acquired from Microbiological Associates, Bethesda, Md.

Table I. (continued)

Medium Employed

All components must be sterile.

Eagle's MEM	77 ml
Agamma calf serum [b]	20 ml
Penicillin-Streptomycin mixture (5000 units each/ml)[a]	2 ml
Glutamine [a]	1 ml
	100 ml

(10 X) GKN Solution

NaCl	80.0 g
KCl	4.0 g
Glucose	10.0 g

Make up to one liter with water, autoclave, and store at 4° C.

Trypsin Solution

Trypsin (Difco 1/250 stock) [c]	2 g	Make a paste in water
(10 X) GKN solution	100 ml	
0.2% Aqueous Phenol Red	6 ml	

Rinse reagents into a one liter volumetric flask and add water to 650 ml. Add enough sodium bicarbonate to give an orange-red color. Shake two hours. Make up to one liter with water, sterilize by filtration and store frozen.

(a) Acquired from Microbiological Associates, Bethesda, Md.
(b) Acquired from Hyland Laboratories, Los Angeles, Calif.
(c) Obtained from Difco Laboratories, Detroit, Mich.

For experimental procedures the concentration of cells in the medium was ascertained by means of an A-O Spencer, bright line hemacytometer, as proposed by Sanford et al. (36). One ml of cell suspension was removed while the suspension was being stirred with a magnetic stir bar. The suspension was added to two ml of sterile 0.02% crystal violet in 0.1 M citric acid solution. This mixture was agitated and a portion of it was placed in both chambers of the hemacytometer. The nuclei appearing in all nine square millimeter areas of each chamber were counted. An average of two countings was made. By a calculation of the dilution factors involved, the cell concentration in the medium was estimated. The suspension was then diluted with more medium to give a final concentration of 900,000 cells/ml.

Preparation of Potassium Pentachlorophenate

PCP (Eastman Organic Chemicals, Rochester, N. Y.) was converted into its potassium salt. Five hundred ml of water was heated to near boiling. One hundred grams of PCP were added in small increments until no more would go into solution. A few drops of 50% aqueous potassium hydroxide were then added and the procedure repeated until all of the potassium pentachlorophenate (KPCP)

was in solution. The solution was then cooled to four degrees centigrade and filtered in a cold Buchner funnel. The crystals were redissolved and recrystalized until the filtrate was free of color. The crystalline KPCP was then dried and stored in the dark.

Inhibitory Dose Studies

On day zero, nine 32 oz prescription bottles were inoculated with 40 ml of cell suspension at a concentration of 900,000 cells/ml. The inoculum for each bottle was removed from a flask as it was constantly stirred by a sterile magnetic bar. The cells were allowed to settle and attach to the glass. On day three, the medium was drained from each bottle and 40 ml of fresh medium was added. Two of the bottles were analyzed on day three for total cell protein as described later. Two bottles were not poisoned in order to provide a control for growth. The five remaining bottles were poisoned with KPCP as follows:

final KPCP concentration	KPCP stock solution	ml of stock solution	total mg KPCP in 40 ml
10 mg/l	8 mg/ml	0.05 ml	0.4 mg
20 mg/l	8 mg/ml	0.10 ml	0.8 mg
40 mg/l	40 mg/ml	0.04 ml	1.6 mg
80 mg/l	40 mg/ml	0.08 ml	3.2 mg
160 mg/l	40 mg/ml	0.16 ml	6.4 mg

On day ten, the cells from the remaining seven bottles were collected for analysis. Each bottle was scraped with a rubber

policeman, decanted, scraped again in 20 ml of 0.85% saline, and rinsed a second time with 20 ml of 0.85% saline. The cell suspension and rinsings were collected in a 90 ml centrifuge tube and centrifuged for ten minutes at 2000 rpm. The supernatant liquid was discarded. The cells were rinsed and centrifuged twice in 20 ml of 0.85% saline. Finally the centrifuged cells were rinsed into a micro-Kjeldahl flask with four rinsings of five ml glass-distilled water and analyzed for total nitrogen.

Tolerated Toxic Concentration

Cells became increasingly resistant to some toxicants over a period of time. The tolerated toxic concentration (TTC), defined by Gablicks (17) as the highest concentration of a toxicant which is tolerated for at least two weeks or for two cell passages, was used to determine the resistance of cells to PCP. To study the tolerated toxic concentration, eight oz. French square bottles were inoculated with ten ml of medium containing 900,000 cells/ml. Each culture bottle was poisoned with one of the following concentrations of KPCP:

final KPCP conc.	KPCP stock solution	ml of stock solution	total mg KPCP in 10 ml medium
20 mg/l	8 mg/ml	0.025 ml	0.2 mg
40 mg/l	8 mg/ml	0.05 ml	0.4 mg
48 mg/l	8 mg/ml	0.06 ml	0.48 mg
60 mg/l	8 mg/ml	0.075 ml	0.60 mg
80 mg/l	8 mg/ml	0.10 ml	0.80 mg
120 mg/l	8 mg/ml	0.15 ml	1.2 mg

On day ten of the experiments, the media from each bottle and the cells in ten ml of trypsin solution were combined and centrifuged. The cell pellet was then dispersed in ten ml of fresh medium by passing repeatedly through the tip of a pipette. A new culture bottle was inoculated with the entire cell pellet in ten ml of medium for those bottles in which cell growth was poor or a fraction of the cell pellet for those bottles in which cell growth was sufficient. At the time of cell passage the bottles were poisoned as before with a fresh solution of KPCP. The cell passage procedure was repeated on day 20 and the growth of the cells was examined on day 30. Also on day 30 the cells from the bottles containing 80 mg/l and 120 mg/l had their medium renewed without KPCP to ascertain their viability. These bottles were observed on day 40.

Growth Experiment Procedures

On day zero, eight 32 oz prescription bottles were inoculated with 40 ml of cell suspension at a concentration of 900,000 cells/ml of medium. The inoculum was stirred continuously during the procedure, and bottles which would subsequently contain control and poisoned cells were alternately inoculated. Four of the bottles were poisoned with either 0.08 ml of 40 mg KPCP/ml or 0.08 ml of 20 mg KPCP/ml to give final concentrations of 80 mg/l or 40 mg/l respectively. The cells were incubated at 23^{o}C for ten days. On day

ten, the medium was drained from the cell layer in each bottle. The cell layer was rinsed two times with 20 ml of 0.85% NaCl. The used media was filtered through glass wool and reserved for further analysis. Rinsings were discarded.

The cells of one bottle were then scraped into ten ml of 0.85% saline with a rubber policeman. The cells were dispersed by passing six times through a siliconized, ten-ml, serological pipette and placed in a siliconized, 100-ml, volumetric flask. The bottle was scraped again in ten ml of 0.85% saline; the cells were again dispersed by six passages through the tip of a pipette and the mixture added to the contents of the volumetric flask. The bottle was rinsed with ten more ml of saline, which was added to the next bottle after it had been twice rinsed free of the medium. This procedure was repeated until the cells from the four control or four poisoned bottles were collected in a 100 ml volumetric flask. Any cells adhering to the triturating pipette were loosened with a fine wire and rinsed into the flask with saline. The flasks were made up to volume with saline and a teflon stirring bar was added.

During rigorous stirring, aliquots of the cell suspension were taken for subsequent analysis with siliconized volumetric pipettes: three ml for a volumetric determination, one ml for a cell count, 20 ml for a protein determination, and 25 ml for dry and ash weight determinations. The remainder of the cell suspension, along with

two ten ml saline rinses of the flask, was poured into a 90 ml centrifuge tube for fatty acid determinations.

Dry and Ash Weight

The 25 ml aliquot was centrifuged ten minutes at 2,000 rpm, and the supernatant liquid was poured off. The centrifuge tube was rinsed (as closely to the pellet as possible without touching it) with glass-distilled water to remove excess salt. The pellet was rinsed four times with two ml of water into a weighed size 00 crucible, dried at 50°C in an oven, and placed in a desiccator over anhydrous $CaSO_4$. The sample was weighed until the weight was constant on a Mettler micro-balance. Ash weight was determined by ashing the sample covered by a crucible lid in a muffle furnace at 600° C for one hour. The crucible was again placed over anhydrous $CaSO_4$ and was weighed to a constant value.

Packed Cell Volume

The three ml aliquot was transferred to a siliconized Bellco graduated centrifuge tube with .0005 ml subdivisions and centrifuged ten minutes at 2300 rpm. The supernatant liquid then was drawn out and blown against the sides of the tube to resuspend any cells not in the capillary. The cells then were centrifuged three hours at 3300 rpm with volume readings taken after each hour.

Cell Counts

The one ml aliquot was placed in a siliconized ten ml volumetric flask and made up to volume with .02% crystal violet in 0.1 M citric acid solution. A small glass stir bar was added, and the contents were agitated by hand and by the stir bar. Portions of the suspension were placed in a bright line, A-O Spencer hemacytometer. The nuclei appearing in all nine square millimeter areas of each of the two chambers of the hemacytometer were counted with a phase-contrast microscope at 200 X magnification. The chambers of the hemacytometer were cleaned and dried and the chambers filled twice more, giving a total of six counts (3 replications x 2 chambers), which were averaged.

Protein

The 20 ml aliquot was placed in a 30 ml micro-Kjeldahl flask along with 100 mg sucrose, 500 mg Na_2SO_4, and 40 mg HgO, using a modification of Colowick and Kaplan's (12) nitrogen determination. The flask was evaporated to dryness in a 60° vacuum oven, and 1.5 ml of concentrated H_2SO_4 were added. The contents of the flask were digested on an electric digestor until the flask's contents had been colorless for half an hour. The cooled digest was washed into a Kjeldahl still with five one ml portions of distilled water. Twenty-

five ml of an aqueous mixture of 10% NaOH and 5% sodium thiosulfate were added to make the digest alkaline. The steam distillate was collected in a 50 ml beaker containing five ml of 5% aqueous boric acid with the condenser tip beneath the liquid level for the first five minutes of distillation and above it for the next two minutes. The tip of the condenser was rinsed with distilled water. The beaker containing the boric acid solution was then titrated to pH 4.2 with 0.01 N potassium biniodate, using a type TTT 1 b Radiometer automatic titrator. Each ml of 0.01 N potassium biniodate was considered equivalent to 0.14 mg of nitrogen, and the mass of protein was estimated by multiplying the nitrogen value by 6.25.

Fatty Acids

The remaining aliquot of the cell suspension was centrifuged ten minutes at 2000 rpm. The supernatant liquid was discarded, and the pellet rinsed into a 100 ml beaker four times with two ml portions of distilled water. Cell lipid was extracted following a method by Bligh and Dyer (5). Twelve milliliters of chloroform and 24 ml of methanol were added to the sample in a 100 ml beaker. The mixture was sonicated 60 seconds at 80% of maximum power with a Biosonik II. The mixture was poured into a 90 ml centrifuge tube and the beaker rinsed into the centrifuge tube three times with four ml portions of chloroform. Twelve ml of water was added to the tube and

the tube was centrifuged five minutes at 2500 rpm. The chloroform layer was drawn off and the water-methanol layer re-extracted twice with eight ml of chloroform. The chloroform layers were evaporated to dryness at 50°C under nitrogen to remove water and the samples stored in chloroform at minus ten degrees centigrade.

The total lipid of serum was similarly extracted to determine the fatty acid content of the medium. Ten milliliters of serum was extracted with the quantities of solvents mentioned above. The residual media from the experiments were also similarly extracted. Twenty-five ml of medium was sonicated with 30 ml $CHCl_3$ and 60 ml MeOH. Then 30 ml of water and 30 ml of $CHCl_3$ were added before centrifugation. The water-methanol layer was re-extracted twice with 20 ml of $CHCl_3$.

Saponification of Fatty Acids

After the solvent had been evaporated, the total lipid extracts were saponified to remove non-fatty acid material. The samples were heated at 80°C for fifteen minutes in a closed, screw cap test tube in four ml of methanol and one ml of 60% aqueous KOH. After cooling two ml of H_2O and five ml of $CHCl_3$ were added, the tube shaken and centrifuged briefly on a clinical centrifuge and the water layer removed to another centrifuge tube. The $CHCl_3$ layer was extracted four more times with two ml of 50% aqueous MeOH. The

soaps were then acidified with four ml of concentrated HCl and the water layer was extracted four times with three ml of hexane. The fatty acids were dried under a nitrogen stream and stored in hexane at minus ten degrees centigrade.

Preparation of Fatty Acid Methyl Esters

The dried fatty acids were heated in a capped test tube at 80°C for ninety minutes in three ml of diethyl ether and three ml of HCl-methanol. After cooling, two ml of water and two ml of hexane were added, mixed, and the combination centrifuged. The aqueous layer was extracted three more times with two ml portions of hexane. The hexane, containing the fatty acid methyl esters (FAME), was then evaporated under a nitrogen stream and the residue taken up and stored in hexane at four degrees centigrade. Portions of the FAME-hexane mixture were injected into the gas chromatograph. Tests using standard fatty acid mixtures resulted in 95% recovery or better of FAME from saponification and methylation procedures.

Methanol-HCl for Methylation

A mixture of 0.5 g iodine, 5.0 g magnesium and 75 ml 100% methanol was refluxed until no more hydrogen was liberated. Anhydrous methanol, 900 ml, was added and refluxing renewed for 30 minutes. Distillation of the anhydrous methanol was carried on with

care to avoid water absorption.

Thirty grams of oven dried NaCl was placed in a 500 ml gas generator bottle to which 80 ml of concentrated H_2SO_4 was added slowly to permit a slow evolution of HCl gas into 100 ml of prepared dry methanol. This process gave anhydrous methanol with an HCl content of 3.8 - 4.6%.

Fatty Acid Standards

Fatty acid methyl esters were obtained from the Hormel Institute, University of Minnesota, Austin, Minnesota. The general method of preparation, according to John D. Nadenicek of the Hormel Institute, was to employ physical techniques such as low temperature crystallization, urea adduct formation, column chromatography, and finally, high vacuum fractionation by distillation. The final products were analyzed at Hormel by gas-liquid chromatography and by thin-layer chromatography as a means of detecting impurities. Structural determinations at Hormel were accomplished when necessary by means of infra-red spectrophotometry and ozonolysis.

Gas Chromatography

Fatty acid methyl esters were analyzed with a Beckman GC-2 gas chromatograph equipped with a hydrogen flame ionization detector. An aluminum column one eighth inch by six feet packed with

ethylene-glycol-succinate coated chromsorb (P) (mesh size 35-80), coated to the extent of 15% by weight, was used in the chromatograph. Oven temperature was 190°C; injection port, 250°C; helium flow rate, 60 ml/minute; sample size, approximately 66 μg in ten μl injected as a solid (28); air flow rate to the detector, 200 ml/minute; and hydrogen flow rate to the detector, 20 ml/minute.

The adequacy of the procedure for separation and quantitative evaluation of fatty acid percentages and masses was checked often with known mixtures of fatty acid methyl esters.

Identification of Fatty Acid Methyl Esters

The FAME (fatty acid methyl esters) were identified by a variety of techniques, each of which was compared with the others so that, with the necessarily small samples, the highest degree of confidence was obtained. The FAME of the control populations were identified and these FAME were matched with those of the day zero medium, day ten media and poisoned populations by retention times and by coincident injection of gas chromatographic samples from two sources.

To verify chromatographic peaks, population FAME samples were injected concomitantly with FAME standards from the Hormel Institute, Austin, Minnesota. The standard FAME used most often were 16:0, 18:0, 18:1 ω9, 18:3 ω3 and 20:0.

Tentative identification of the saturated, branch chain and unsaturated FAME was accomplished by plotting the log of the retention times against the number of carbons in the chain (2, 14, 34). For fatty acids with the same number of double bonds (homologous series) the graphs of log retention against the number of carbon atoms gave straight lines and allowed the tentative establishment of the number of carbons and the number of double bonds for FAME contributing to the chromatographic record. Standard saturated FAME from the Hormel Institute were used to establish the proper carbon number to peaks of matching retention times. The technique of semi-log plotting was also used to check results from silver nitrate separations, hydrogenations and the products of ozonolysis.

Separation of Fatty Acid Methyl Esters by Thin-Layer Chromatography

Plates for separation of the FAME were made using 6.25 g of silver nitrate, 25 g of Mallinckrodt silicic acid mesh size 200 and less, and 50 ml of water. Eight by eight inch, thin layer plates were spread at 250 μ thickness, or less, of the mixture, oven dried at 110^{o}C for one hour and used the same day (31).

Samples of FAME were spotted in a nitrogen atomosphere to prevent oxidation, developed with 20 percent diethyl ether in hexane for 25 minutes and detected with 2, 7-dichlorofluorescein spray

visualized under ultraviolet light. The FAME formed bands related to their degree of unsaturation, saturated FAME being the greatest distance from the original spots and fatty acids with five and six double bonds being nearest the original spot. The bands of FAME were scraped from the plate separately and extracted into chloroform. A portion of the FAME from each band was hydrogenated. Another portion was subjected to reductive ozonolysis.

Hydrogenation of the Fatty Acid Methyl Esters

The fractions of FAME removed from the bands on silver nitrate plates were hydrogenated by the method of Farquhar (15). The FAME sample was dissolved in a small amount of hexane and subjected to a hydrogen atmosphere for 30 minutes in the presence of Adam's platinum oxide catalyst.

After hydrogenation, the sample was filtered to remove catalyst and injected into the gas chromatograph for identification of the saturated FAME produced. Hydrogenation converts unsaturated FAME to saturated FAME of the same carbon chain length and thus by percentage composition analysis of the sample before and after hydrogenation and by comparisons of the retention times of the products with standard FAME, the number of carbons can be ascertained for each of the original unsaturated FAME.

Ozonolysis of the Unsaturated Fatty Acid Methyl Esters

Location of the ethylenic groups of the unsaturated FAME were investigated by reductive ozonolysis. An apparatus modified after Bonner (6) was used to generate ozone. The unsaturated FAME fractions separated on silver nitrate plates were dissolved in one ml of dichloromethane in a test tube, cooled to -65°C in a dry ice-acetone bath and ozone was bubbled into the dichloromethane through a disposable pipette. Ozonolysis required approximately five minutes and completion of the ozonolysis could be detected by the odor of ozone at the mouth of the tube.

The ozonide formed from each unsaturated FAME, on triphenylphosphine reduction, yielded an aldehyde and an aldehyde-ester. The aldehyde and aldehyde-ester portions were subjected to gas chromatographic analysis at oven temperatures of 100°C and 160°C respectively. Standard FAME, 18:1 ω9 and 18:1 ω6, from the Hormel Institute, were subjected to the same procedure and the resulting aldehydes and aldehyde-esters were used as standards in the gas-chromatographic analysis.

The amounts of individual FAME present in a mixture are proportional to the product of peak height by the retention time of the FAME (4, 7, 8, 14). The peak height by retention time products were totaled and the fractional contribution of each fatty acid to the

total was computed. Thus was obtained the percentage contribution of each fatty acid to the total fatty acids in the sample. The mass of individual FAME was determined by the product of its percent contribution and the total FAME mass.

Statistical Analysis

Numerical data obtained in this study were processed by the Computer Center, Department of Statistics, Oregon State University. Means, standard deviations, regression analyses and simple coefficients of variations were computed.

Abbreviations

Several concepts will be discussed which require an extensive number of words in explanation. In order to envisage some of these concepts more clearly, several abbreviations have been used. These abbreviations are listed and defined below.

AW	ash weight
BSS	Earl's balanced salt solution
DW	dry weight
FA	fatty acids
FAME	fatty acid methyl esters
GKN	glucose, potassium, sodium solution
GLC	gas-liquid chromatography

ID	inhibitory dose
KPCP	potassium pentachlorophenate
MeOH	methanol
MEM	Eagle's minimum essential medium
N	derived Kjeldahl nitrogen; number of replications
NFOM	non-FAME organic matter. Calculated as the mass of total organic matter minus the mass of FAME.
p	the probability that the two populations of numbers compared are not different populations, but are members of the same population of numbers
PCP	pentachlorophenol
pg	picograms
TLC	thin-layer chromatography
TOM	total organic matter. Calculated as the difference between dry weight and ash weight.
TTC	tolerated toxic concentration
Vol 1	packed cell volume of cells after one hour of centrifugation
Vol 2	packed cell volume after two hours of centrifugation
Vol 3	packed cell volume after three hours of centrifugation
<	less than

DATA AND DISCUSSION

Data related to toxic and lethal levels of KPCP will be presented first. Then data on population growth will be given and data on cell properties will be noted. To avoid repetition, discussion and comparison with the literature will be provided as the data are presented.

Tolerated Toxic Concentration

The tolerated toxic concentration (level of KPCP poisoning at which cells remained viable through two cell passages and 30 days of incubation) was at least 80 mg KPCP per liter and less than 120 mg KPCP per liter. After 30 days of culturing and two cell passages, the cells exposed to 80 mg KPCP/l were still viable although growth was poor. The cells were not viable after being exposed to 120 mg KPCP/l under the above conditions, and no cells were visible growing on the glass of the culture bottle after 30 days.

Data from the inhibitory dose studies had indicated that net growth of the cells at 80 mg KPCP/l was approximately 30% of control net growth, while at 120 mg KPCP/l net growth was approximately zero. This would indicate that little or no resistance to KPCP developed in the cell cultures between seven days and 30 days of exposure to KPCP. Thirty percent of control net growth in this

culture is apparently sufficient to maintain the culture and make up for losses developing during the trauma of handling in reculturing.

Inhibitory Dose

The inhibitory dose of potassium pentachlorophenate (KPCP) was determined graphically using total nitrogen as a measure of growth. The net growth of the cells over seven days, when measured as milligrams of nitrogen per culture bottle, is a linear function of KPCP concentration as shown in Figure 1. Net growth is here defined as the amount of nitrogen in the cells on day ten that is in excess of the amount in the cells on day three. A regression analysis of the data presented in Figure 1 indicates that the slope of the line is - 0.018 mg of nitrogen per mg of KPCP per liter with a standard error of 0.0006 and the y-intercept is 2.34 mg of nitrogen with a standard error of 0.045.

The formula for the regression line in Figure 1 is:

$$\text{Nitrogen Increase} = A + B \cdot [KPCP] \text{ or } dN = A + B \cdot C \quad (1)$$

Where $A = 2.337$ mg of nitrogen

$B = -0.018$ mg of nitrogen per mg of KPCP per liter

Standard error of $A = 0.045$

Standard error of $B = 0.0006$

and where dN equals the mg of N in the cell population on day ten above day three, A equals the amount of N in the absence of KPCP,

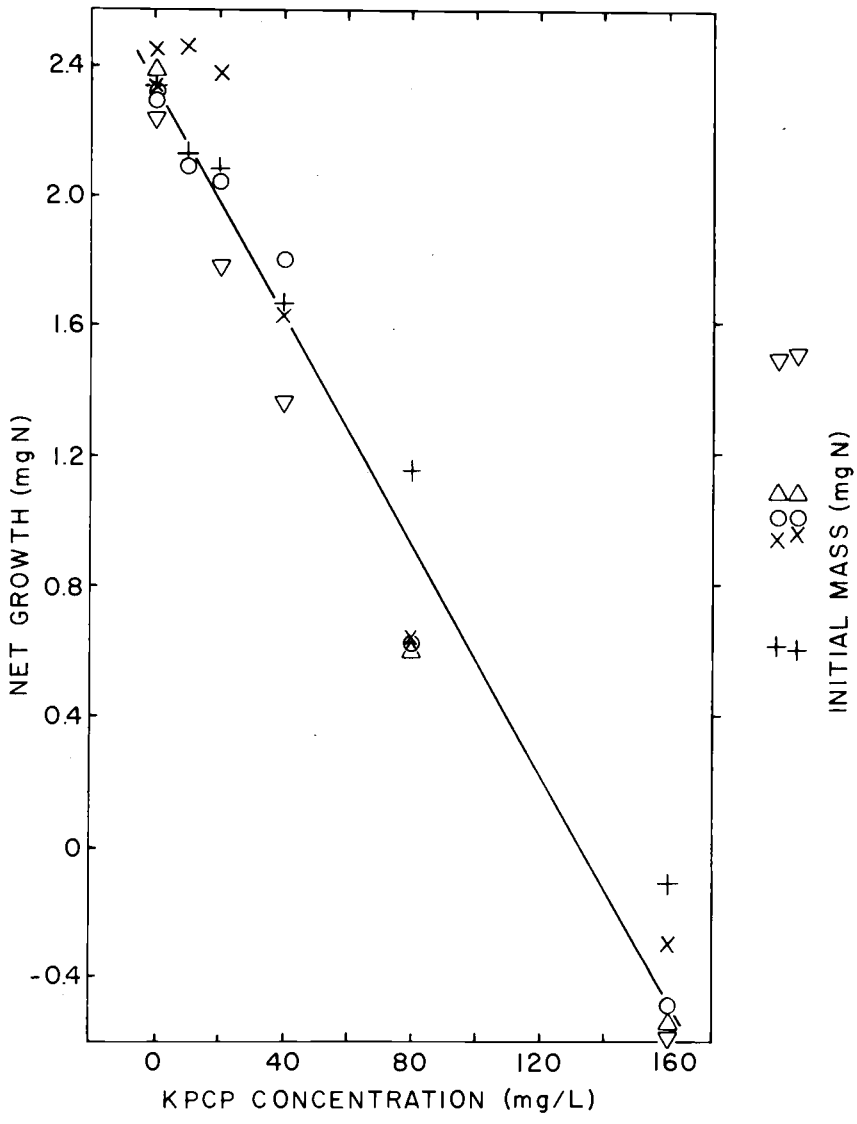


Figure 1. Net growth in cell populations exposed to potassium pentachlorophenate. Each symbol represents the net growth in one culture bottle between day three and day ten. Like symbols represent values from the same experiment. Symbols to the right of the rectangle represent the initial masses (day three nitrogen mass per culture bottle) of each experiment.

B is the change per unit of KPCP, and C is the concentration of KPCP.

Therefore, Net Growth = dN = 2.337 - 0.018 [KPCP] (2)

The fraction of control growth, attained by a poisoned population, is given by the fraction in equation 4, and the fractional reduction is given by the parenthesis in equation 4. Hence, the formula for percent inhibition of growth in the presence of KPCP would be:

$$\% \text{ inhibition} = \frac{2.337 - \text{Net Growth}}{2.337} \times 100 \quad (3)$$

$$= \left(1 - \frac{\text{Net Growth}}{2.337}\right) \times 100 \quad (4)$$

The KPCP concentration at a given increase in nitrogen on day 10 can be obtained from formula two. Substitution of formula (2) in formula (3) gives

$$\% \text{ Inhibition} \times 1.30 = C = [KPCP] \quad (5)$$

Thus, the 20% inhibitory dose (ID_{20}) would be 27 mg of KPCP per liter; the ID_{50} or the dose giving 50% inhibition would be 66 mg KPCP/l; and the dose to prevent growth without any increase in population size would be 133 mg KPCP/l.

Although an attempt was made to inoculate the bottles of each experiment at a cell concentration of 900,000 cells/ml, the day three samples (Initial mass, Figure 1) had extremes between experiments of 0.6 and 1.5 mg of nitrogen as indicated at the right of Figure 1. Even so, all of the points for the net growth in non-poisoned cells are

clustered with extremes between experiments of 2.23 and 2.45. Those experiments which began with a high initial (day three) nitrogen mass produced less net growth than those experiments with a low initial mass. Therefore; within the range of these experiments, net growth is an inverse function of initial mass. Density related growth inhibition is a common phenomenon in monolayer cell cultures.

It should be emphasized that the data presented in Figure 1 are plotted in linear ordinates and linear abscissae and indicate a linear relationship between dose and effect instead of the more frequent logarithmic or sigmoid relationships. Note that where duplicate samples were analyzed (day three samples and day ten control samples) the two points are very close, indicating that the method of protein measure is reproducible.

Growth of Cultures of Salmonid Embryonic Tissue

Figure 1 indicates that potassium pentachlorophenate (KPCP) at 20 mg/l had a measurable effect on growth as measured by nitrogen incorporation, but clear-cut effects were not established until 40 mg/l were used. Hence, to study the effect of KPCP on some of the metabolic changes during growth, KPCP concentrations of 40 mg/l and 80 mg/l in the media were chosen. The data collected will be developed first with reference to control populations,

next to populations exposed to 40 mg/l, and then to populations exposed to 80 mg of KPCP/l.

The general descriptions of some parameters of growth in control populations are contained in Tables II-V. After some details of description have been developed, the reproducibility of the measurements will be discussed, then cell fragility, nitrogen content of protein, generation time, and finally, material apportionment between cells and media. The same order will be used in the development of data from poisoned populations. Finally, a general discussion of the data on growth will be given and will be related to information in the literature.

Control Populations

Population growth over a ten day period can be evaluated from the measurements of day ten cell numbers, volume, dry weight, ash weight, nitrogen content and total FAME (fatty acid methyl esters). Table II contains data for control populations averaged from 19 growth experiments. The values for day zero are calculated from day ten values on the basis of 144×10^6 cells (distributed to four sample bottles; four bottles x 40 ml of medium x 900,000 cells per ml = 144,000,000 cells) inoculated on day zero. Standard deviations of the data are also provided in Table II. The standard errors of the means can be obtained by multiplying the standard deviation by

Table II. Means and standard deviations of growth parameters for 19 control cell populations.

	Symbol	Units	Day 0	Day 10	Standard Deviation % of Mean	10 Day Growth Day 10 Minus Day 0
Cell Count		millions	144	339 ± 62	18	195
Population Volume 1 hr packing	Vol 1	mm^3	261	614 ± 88	14	353
Population Volume 2 hr packing	Vol 2	mm^3	218	514 ± 127	25	296
Population Volume 3 hr packing	Vol 3	mm^3	145	341 ± 109	32	196
Dry Weight	DW	mg	27.0	63.6 ± 4.6	7	36.6
Ash Weight	AW	mg	3.24	7.63 ± 1.02	13	4.39
Nitrogen	N	mg	4.00	9.4 ± 0.64	7	5.41
Total Fatty Acid Methyl Esters	FAME	mg	2.93	6.88 ± 0.65	10	3.96
Total Organic Matter	TOM	mg	23.8	56.0 ± 3.8	7	32.2
Non-Fatty Acid Organic Matter	NFOM	mg	20.9	49.1 ± 3.4	7	28.2

Table III. Means and standard deviations of growth parameters for cell populations exposed for ten days to 40 mg/KPCP, their paired controls and the differences between pairs.

	Units	Controls N = 6	Poisoned 40 mg/l KPCP N = 6	Differences Poisoned Minus Controls N = 6
Cell Count	millions	307 ± 69	326 ± 61	18 ± 39
Population Volume 1 hr packing	mm^3	587 ± 89	619 ± 44	32 ± 54
Population Volume 2 hr packing	mm^3	417 ± 143	442 ± 116	25 ± 39
Population Volume 3 hr packing	mm^3	272 ± 83	280 ± 64	8 ± 44
Dry Weight	mg	63.9 ± 3.8	60.7 ± 2.5	-3.2 ± 2.2**
Ash Weight	mg	7.76 ± 0.78	7.68 ± 0.32	-0.08 ± 0.64
Nitrogen	mg	9.35 ± 0.57	9.02 ± 0.30	-0.33 ± 0.54
Total Fatty Acid Methyl Esters	mg	6.61 ± 0.33	6.26 ± 0.31	-0.35 ± 0.32*
Total Organic Matter	mg	56.2 ± 3.2	53.0 ± 2.4	-3.1 ± 1.7***
Non-Fatty Acid Organic Matter	mg	49.6 ± 2.9	46.8 ± 2.3	-2.8 ± 1.5***

*Significant at $p<.05$; **Significant at $p<.01$; ***Significant at $p<.005$

Table IV. Means and standard deviations of growth parameters for populations exposed for ten days to 80 mg KPCP/1, their paired controls and the differences between pairs.

	Units	Control 80 mg KPCP/1 N = 7	Poisoned 80 mg KPCP/1 N = 7	Differences Poisoned-Control N = 7
Cell Count	millions	325 ± 57	204 ± 17	-121 ± 49***
Population Volume 1 hr packing	mm^3	569 ± 76	420 ± 63	-148 ± 86***
Dry Weight	mg	60.4 ± 4.1	39.8 ± 2.7	-20.6 ± 5.3***
Ash Weight	mg	7.21 ± 0.97	5.36 ± 0.48	-1.85 ± 1.14***
Nitrogen	mg	9.06 ± 0.70	6.07 ± 0.40	-3.00 ± 0.75***
Total Fatty Acid Methyl Esters	mg	6.68 ± 0.86	5.10 ± 0.52	-1.58 ± 0.64***
Total Organic Matter	mg	53.2 ± 3.4	34.5 ± 2.3	-18.8 ± 4.4***
Non-Fatty Acid Organic Matter	mg	46.6 ± 2.9	29.4 ± 2.1	-17.2 ± 4.0***

*Significant at $p < .05$; ***Significant at $p < .005$

Table V. Means and standard deviations of growth parameters for six cell populations poisoned with 80 mg/l KPCP and their paired controls, seven poisoned populations and their paired controls, and a combination of 15 poisoned populations.

	Units	Controls N = 6	Poisoned N = 6	Controls N = 7	Poisoned N = 7	Poisoned N = 15
Cell Count	Millions	387 ± 35	195 ± 40	325 ± 57	204 ± 17	206 ± 33
Population Vol 1	mm^3	694 ± 43	416 ± 48	569 ± 76	420 ± 63	428 ± 60
Population Vol 2	mm^3	633 ± 44	191 ± 64	494 ± 78	251 ± 140	228 ± 112
Population Vol 3	mm^3	444 ± 98	110 ± 16	311 ± 74	132 ± 36	127 ± 33
Dry Weight	mg	67.1 ± 3.5	38.5 ± 4.8	60.4 ± 4.1	39.8 ± 2.7	40.2 ± 4.8
Ash Weight	mg	7.99 ± 1.26	5.35 ± 1.66	7.21 ± 0.97	5.36 ± 0.48	5.42 ± 1.06
Nitrogen	mg	9.86 ± 0.39	5.82 ± 0.60	9.06 ± 0.70	6.07 ± 0.40	6.08 ± 0.61
FAME	mg	7.40 ± 0.28	6.18 ± 1.19	6.68 ± 0.86	5.10 ± 0.52	5.59 ± 0.95
TOM	mg	59.1 ± 2.5	33.1 ± 3.7	53.2 ± 3.4	34.5 ± 2.3	34.8 ± 4.2
NFOM	mg	51.7 ± 2.4	27.0 ± 3.0	46.6 ± 2.9	29.4 ± 2.1	29.2 ± 4.0

0.229 ($1/\sqrt{19}$).

The reproducibility of measurement of the parameters can be pictured from the ratios of standard deviations to the means. On this basis standard nitrogen and dry weight determinations are both precise measurements. The calculated total organic matter (TOM) and non-FAME organic matter (NFOM) had precisions of measurement similar to that of dry weight.

Following nitrogen, dry weight, TOM and NFOM, in order of decreasing precision, are total FAME, one hour volume and ash weight, cell counts, two hour volume, and three hour volume. It is possible that the packed cell volumes measured after two and three hours of centrifugation are more variable than the measures after one hour because significant numbers of cells break with the stress of prolonged centrifugation. The measurement after the first hour of centriguation seems the best indicator of packed cell volume, while the reduction in the packed mass with the second and third hours of centrifugation can be used as an indication of cell fragility. A decrease in volume and an increase in variability are prominent in the second and third hour packed cell volumes of cells poisoned with 80 mg KPCP/l (Table V).

If one examines the nitrogen and non-fatty acid organic matter contents of either control or poisoned populations, one finds that the non-fatty acid organic matter mass is always less than 6.25 times

the nitrogen content. Since carbohydrate and non-fatty acid moieties of phospholipid should be components of the non-fatty acid organic matter, the nitrogen content of these populations must be equivalent to more than 16 percent of the protein mass. The nitrogen values in the control populations, as percents of non-fatty acid organic matter, were 18.9% and 19.4%.

The number of cells in the control populations increased from 144 million to 339 million over the ten day period. If a doubling of the number of cells is taken to represent the production of one generation, then the population had increased to 2.4 times the day zero value, 1.24 generations had been produced, and the average generation time was 194 hours. Pilcher et al. (30), using 20 percent dialyzed calf serum, found the generation time of cultures of CSE 119 during most rapid growth to be 50 hours; but over a ten day growth period an average generation time of 97 hours can be calculated from their data. The 194 hour generation time was calculated, for the experiments presented here, over the ten day period and not for the period of most rapid growth.

Effects of Pentachlorophenol

Table III presents growth data of cultures exposed to 40 mg KPCP/l and their paired controls. Those parameters which showed significant differences by Student's T test between the growth of

control and poisoned cells were dry weight, FAME, total organic matter, and non-FAME organic matter. There was no statistically significant difference in the number of cells produced in ten days or of the total cell volume. The percentage reductions of volume with two and three hours of centrifugation were nearly identical for control and poisoned cells. This would seem to indicate that there was no increased fragility in the poisoned cells.

The ash and nitrogen content of populations grown in control media and in media with 40 mg KPCP/l did not differ significantly. The protein equivalent of the nitrogen loss, 1.72 mg protein (using the average 19.1 percent nitrogen for protein as derived from control cells), would also be statistically non-significant.

The loss of non-fatty acid organic matter was 2.8 mg; therefore, as nitrogen did not change significantly, organic material other than protein may have been present in lower concentration in the poisoned cells than in control cells.

In summary, the differences between control populations and populations poisoned with 40 mg KPCP per liter (Table III) with respect to cell number, population volume, ash, and nitrogen, were not statistically significant. Poisoned populations contained 3.2 mg less dry matter than control populations, 0.35 mg less fatty acid (FAME), 3.1 mg less total organic matter and 2.8 mg less non-fatty organic matter.

Populations poisoned with 80 mg KPCP per liter differed from paired control populations in all parameters estimated (Tables IV-VI). All of the differences were statistically significant. The reduction in cell numbers was proportionately greater than the reduction in volume indicating an increased average cell volume in poisoned cells.

If the nitrogen is equivalent to 6.25 times cellular protein, the three mg reduction in cellular nitrogen would more than account for the 17.2 mg reduction of non-fatty acid organic matter. Nitrogen contents of control populations, as percent of non-fatty acid organic matter, were 18.9 and 19.4% (average 19.1%). In the poisoned populations, they were 19.3% and 20.6%. KPCP poisoning may reduce the content of non-protein, non-fatty acid organic matter, or may cause a proportionately higher catabolism of low nitrogen protein than of protein of higher nitrogen content. At 19.1% of nitrogen in protein, a reduction of three mg in dry matter is equivalent to 15.7 mg of protein. Comparing 15.7 with 17.2 mg suggests a slight loss in the production of non-protein organic matter under the action of KPCP.

Tables V and VI present growth data from two sets of experiments with populations exposed to 80 mg KPCP/1, their paired controls and a combination of the two sets of experiments. The two sets of experiments with 80 mg KPCP/1 were segregated because they were separated in time by six months and the cell medium,

Table VI. Mean differences and standard deviations of growth parameters between six populations poisoned with 80 mg/l KPCP and their paired controls, seven poisoned populations and their paired controls, and a combination of the differences between the 13 poisoned populations and their paired controls.

	Units	Poisoned minus Controls N = 6	Poisoned minus Controls N = 7	Poisoned minus Controls N = 13
Cell Count	millions	-192 ± 38***	-121 ± 49***	-154 ± 56***
Population Volume 1 hr packing	mm^3	-278 ± 58***	-148 ± 86***	-208 ± 98***
Population Volume 2 hr packing	mm^3	-443 ± 38***	-243 ± 107***	-335 ± 131***
Population Volume 3 hr packing	mm^3	-334 ± 101***	-180 ± 56***	-251 ± 110***
Dry Weight	mg	-28.6 ± 5.9***	-20.6 ± 5.3***	-24.3 ± 6.8***
Ash Weight	mg	-2.64 ± 1.02***	-1.85 ± 1.14***	-2.21 ± 1.12***
Nitrogen	mg	-4.04 ± 0.58***	-3.00 ± 0.75***	-3.48 ± 0.85***
Total Fatty Acid (methyl esters)	mg	-1.22 ± 1.38*	-1.58 ± 0.64***	-1.42 ± 1.02***
Total Organic Matter	mg	-26.0 ± 5.3***	-18.8 ± 4.4***	-22.1 ± 5.9***
Non-Fatty Acid Organic Matter	mg	-24.8 ± 4.2***	-17.2 ± 4.0***	-20.7 ± 5.5***

*Significant at $p<.05$; ***Significant at $p<.005$

after depletion, was not examined for fatty acids in the first group. The populations poisoned at 80 mg KPCP/l had fewer cells, a smaller population volume and less of each chemical constituent estimated. Growth was most definitely inhibited by 80 mg of KPCP/l. The packed cell volume was greatly reduced during the second and third hours of centrifugation.

Table VI includes data on the differences between the poisoned populations and their paired controls for the two sets of experiments, using 80 mg KPCP/l, separately and combined. The differences recorded in the last column of the table would have occurred by chance with a probability less than 0.005.

The 144 million cells inoculated on day zero increased to 206 million cells in ten days in populations exposed to 80 mg KPCP/l (Table V). The day ten count was significantly less in the populations exposed to 80 mg KPCP/l than in paired control populations (Table VI); but exposure to 40 mg KPCP/l did not affect the day ten count (Table III). The populations exposed to 80 mg KPCP/l in ten days produced cell numbers averaging 1.4 times the original population, indicating an average of 0.49 generations and a net generation time of 490 hours. Cells exposed to 80 mg KPCP/l used considerably longer times between successive cell divisions than the average 194 hours for control cells and cells exposed to 40 mg KPCP/l.

Cell populations exposed to 80 mg KPCP/1 had significantly less volume with one hour of packing than did their paired controls (Table VI). This difference increased with two hours of centrifugal packing and increased further, on a percentage basis, with three hours of packing. The faster rate of centrifugal packing for populations poisoned with 80 mg KPCP /1 over the rate for paired control populations indicates a more fragile plasma membrane in cells exposed to 80 mg KPCP/1. Populations exposed to 40 mg KPCP/1 packed similarly in the centrifuge to their paired controls and thus these cells did not have more fragile membranes.

The cell populations exposed to 80 mg KPCP/1 contained significantly less dry matter, ash, nitrogen, total fatty acid, organic matter and non-fatty acid organic matter than did their paired controls (Table VI). The losses due to poisoning with 80 mg KPCP/1 were, for each parameter measured, greater than losses due to poisoning with 40 mg KPCP/1 (Tables III and VI).

Material Distribution: Media and Cells

The masses of dry matter, ash and derived fatty acid methyl esters in some media were determined after ten days growth of control populations and populations exposed to 40 and 80 mg KPCP/1. Day zero values for populations were based on day zero cell counts which were also used to make proper cell dilution to 900,000 cells/ml in the inoculum. Some day zero values for media were determined directly.

Control Populations

The 160 ml of medium which was used to support the growth of control and poisoned cell populations contained 3,749 mg of dry matter on day zero. From day zero dry matter, 16.5 mg of fatty acid methyl esters (FAME) and 1,502 mg of ash were derived. When components of the cells were added to the components in the medium, day zero values became 3,776 mg of dry matter, 19.4 mg of FAME and 1,505 mg of ash (Table VII).

During ten days growth of control populations, a statistically significant 2.8 mg (six experiments) of FAME was lost from medium plus cells. The cells of control populations presumably used fats

Table VII. Mean masses (mg) and standard deviations of dry matter, derived fatty acid methyl esters and ash in cultured cells plus their 160 ml of medium.

	Day 0	Control Day 10	Poisoned 40 mg KPCP/l Day 10	Control Day 10	Poisoned 80 mg KPCP/l Day 10
Medium					
Dry Matter	3,749	3,724	3,720	3,740	3,741
Derived FAME	16.5	10.0	9.6	10.1	12.0
Ash	1,502	1,497	1,496	1,507	1,507
Medium plus Cells					
Dry Matter	3,776	3,788 ± 69	3,781 ± 25	3,801 ± 10	3,782 ± 17
Derived FAME	19.4	16.6 ± 0.7	15.8 ± 1.4	16.8 ± 2.1	17.1 ± 2.2
Ash	1,505	1,504 ± 22	1,503 ± 21	1,515 ± 18	1,513 ± 11
Replications		6	6	7	7

from the medium as an energy source. The changes in dry matter and ash for medium plus cells were not statistically significant. In seven later experiments the growth of the seven control populations was accompanied by a statistically significant increase of 25 mg in dry matter of cells plus medium, and a loss of 2.6 mg of FAME. The change in ash was not statistically significant. The difference between the first set of control experiments and the second set is that the controls of the second set showed statistically significant increases in the quantities of dry matter over a ten day period. The raw data indicate that there was an increase in dry matter in most of the earlier controls, but that the high standard deviation of the ten day mass of dry matter in the medium was due to one exceptionally low value.

Poisoned Populations

The total amount of dry matter and ash (medium plus cells) was not altered between day zero and day ten in populations poisoned with 40 or 80 mg KPCP/l. There was a disappearance of 3.6 mg in FAME with 40 mg/l, but only 2.3 mg with 80 mg/l. Thus the growth of populations poisoned with 40 or 80 mg KPCP/l induced the disappearance of fatty acids from the media.

Control vs Poisoned Cells

The depleted media of six cell populations poisoned with 40 mg KPCP/1 averaged 4 mg less dry matter, 0.43 mg less FAME and 1 mg less ash than the media of their paired control populations. The differences between depleted control and poisoned cell media were not statistically significant. The differences for total dry matter, FAME and ash (cells plus medium) between control and poisoned populations also were not statistically significant.

The media depleted by cell populations poisoned with 80 mg KPCP/1 averaged 1.9 ± 1.5 mg more FAME than did control media ($p < 0.01$). The populations exposed to 80 mg KPCP/1 did not remove as much FAME from the media as did control populations, probably because the poisoned populations were much smaller than control populations. Total dry matter (medium plus cells) was not altered by cell cultures during growth under the influence of 80 mg KPCP/1, but was increased in control cultures. Total ash (medium plus cells) was not significantly different between day zero and day 10 in either poisened or control cultures.

Discussion

Pentachlorophenol *in vitro* interferes with oxidative phosphorylation (37). This action can explain its lethal effects at higher

concentrations and its interference with growth under a controlled food intake as well as a decreased efficiency in converting food calories to tissue calories. Chapman found that five gram cichlid fish suffered no fatalities when exposed to 0.2 mg KPCP/1 for 30 days, but that none survived 48 hours of exposure to 0.4 mg/1. Interference with cichlid growth and caloric conversion efficiency was noted with 0.2 mg/1 (9). Pentachlorophenol also caused a decrease in the growth of cultures of embryonic cells from coho salmon, but the minimum concentration which established a definite effect was 40 mg/1; 133 mg/1 would suppress all growth and represents a close approximation to the maximum tolerated concentration. The difference in apparent sensitivity between cells and cichlid fish probably was partly due to proteins in the media reducing the concentration of free pentachlorophenol.

Data presented here indicate a linear relationship between concentration of KPCP and effect. Chapman (10) noted a similar linear relationship with salmonid alevins. Growth of alevins was reduced by about six percent for each ten part per billion increase in sodium pentachlorophenate concentration. It would be worthwhile investigating the meaning of these linear relationships.

During ten day growth of cultures of embryonic cells from coho salmon, no statistically significant increase in dry matter content of media plus cells occurred when 40 mg/1 or 80 mg KPCP/1

were present. However, there was a statistically significant increase in this dry matter content for control populations.

Both control and poisoned cell populations removed fatty acids from the medium. Control populations and populations poisoned by 40 mg KPCP/l removed similar amounts of fatty acids from the medium. A smaller amount was removed by cells poisoned by 80 mg KPCP/l, presumably because the populations were smaller. Control cell populations and populations poisoned by 40 mg KPCP/l had similar fatty acid contents after ten days of growth. Again partly because the cell populations were smaller, the populations growing ten days in the presence of 80 mg KPCP/l contained smaller quantities of fatty acids with statistical significance.

The disappearance of fatty acids was to be expected, but an increase in dry matter was a surprise. However, each peptide bond broken can increase the molecular weights of the products by the molecular weight of water. Further, the development of many citric acid cycle substrates, especially if from fatty acids, requires the incorporation of oxygen and this also increases the weight of the resulting molecules.

While the disruption of peptide bonds would cause an increase in dry weight, the available evidences (in cell lines where it has been investigated) suggests that no significant amount of protein from the medium is hydrolyzed to provide a source of amino acids (13, 27).

Most cultured animal cells require the presence of proteins in the medium. The function of the protein is not to provide amino acids. This is evident from experiments with isotopically labelled serum protein, and from the fact that exogenous amino acids are essential for growth.

The protein factor or factors have not been clearly identified, and the mechanism of their action is also unknown. One function of the protein is to promote attachment of cells to glass. This flattening factor is apparently an α-globulin. The molecular species of protein which embodies the growth-promoting factor(s) may be concentrated in a small subfraction of the α-2-globulin. Possibly growth promotion is due not to the protein, but to small molecular weight components which are either liberated from or produced from protein in the course of its proteolytic degradation (27).

Serum protein is utilized by the cell only to a very limited degree for protein synthesis (27). The magnitude of the utilization leaves serious doubt as to whether the cell possesses the enzymatic mechanisms for degrading exogenous protein to amino acids.

Since the production of amino acids from proteins does not seem likely to explain the increase in dry weight of medium plus cells in control cultures, the incorporation of oxygen requires investigation. Since molecular oxygen primarily picks up hydrogen and forms water at the end of the electron transport system, some other

source of incorporation of oxygen is required. Several animal cell cultures have been shown to fix CO_2 from the nutrient medium, and to require it as a nutrient factor. CO_2 fixation hasn't been explored in these cells, but data have been collected at Oregon State University indicating that CSE-119 requires carbon dioxide for growth. At the end of the ten day growth period, some of the CO_2 from bicarbonate in the medium would presumably have been converted into cellular components, and this CO_2 would not be removed during the dry weight determination. Hence it might contribute to an increase in total dry weight.

Summary

Under the influence of 40 mg KPCP/l, the dry weight, fatty acid content and organic matter of cultures of salmonid embryonic cells after ten days growth in Eagle's essential medium were reduced. At 80 mg/l, cell count, population volume, dry weight, ash, nitrogen, fatty acids and organic matter of the cell population were reduced. The reduction in growth was related linearly to the concentration of KPCP. Total dry matter in cells plus media was not altered during growth under the influence of 80 mg KPCP/l, but was increased in control cultures. Both control and poisoned cell populations removed fats from the media.

Characteristics of Average Cells in Salmonid Embryonic Cell Cultures

On passage of cells after ten days of growth, fresh media were inoculated with 900,000 cells per ml of medium; 40 ml of medium was present in each bottle and four bottles were used for the population. Immediately after cell passage the new population contained 144,000,000 cells (900,000 cells per ml x 40 ml x 4 bottles). Composite samples from four bottles were required to give reasonable accuracy in the determination of the mass of dry weight, nitrogen, ash and fat after ten days growth.

The previous section discussed some of the characteristics of control and poisoned populations in cultures of cell line CSE-119 developed from salmonid embryonic cells. On the assumption that the cells of a population are similar to each other, the characteristics of of the populations depend on the characteristics of single cells and on the number of cells in the population. Under the action of 80 mg of potassium pentachlorophenate (KPCP) per liter cell numbers were reduced, but dry matter, nitrogen, fat and ash were not reduced proportionally. Hence the characteristics of the average cell were changed. Data on the characteristics of average cells in control populations and populations poisoned with 40 mg KPCP/1 or 80 mg KPCP/1 are given in Tables VIII, IX, and X.

Table VIII. Cell characteristics after ten days growth. Means and standard deviations of cell parameters from control populations, populations poisoned with 80 mg/1 KPCP and populations poisoned with 40 mg/1 KPCP with their paired controls.

	Units	Control Populations N = 19	Poisoned Populations 80 mg/1 KPCP N = 15	Control Populations N = 6	Poisoned Populations 40 mg/1 KPCP N = 6
Vol 1/Cell	$1x10^{-12}$ ml	1873 ± 438	2098 ± 282	2004 ± 562	1965 ± 418
Vol 2/Cell	$1x10^{-12}$ ml	1551 ± 442	1121 ± 550	1409 ± 567	1374 ± 359
Vol 3/Cell	$1x10^{-12}$ ml	1026 ± 328	623 ± 145	921 ± 326	881 ± 231
DW/Cell	pg	194 ± 44	197 ± 20	220 ± 65	192 ± 37
AW/cell	pg	23.4 ± 6.1	26.8 ± 5.9	26.6 ± 7.9	24.3 ± 4.4
N/Cell	pg	28.7 ± 5.9	29.9 ± 3.5	32.0 ± 8.2	28.6 ± 5.5
FAME/Cell	pg	21.0 ± 4.6	27.4 ± 4.5	22.7 ± 6.7	19.8 ± 3.8
TOM/Cell	pg	171 ± 39	170 ± 16	193 ± 57	168 ± 33
NFOM/Cell	pg	145 ± 26	141 ± 16	161 ± 37	143 ± 29

Table IX. Mean differences of cell characteristics and standard deviations of differences. Differences are given between cell populations poisoned with 40 mg/1 KPCP and their paired controls, cell populations poisoned with 80 mg/1 KPCP and their paired controls. Data are given for six early studies, seven later studies, and a combination of the 13 populations poisoned with 80 mg/1 KPCP and their paired controls.

	Units	Poisoned-Control 40 mg/1 KPCP N = 6	Early Experiments Poisoned-Control 80 mg/1 KPCP N = 6	Later Experiments Poisoned-Control 80 mg/1 KPCP N = 7	Combined Poisoned-Control 80 mg/1 KPCP N = 13
Vol 1/Cell	μ^3	-39 ± 371	368 ± 172***	252 ± 271*	305 ± 230***
Vol 2/Cell	μ^3	-35 ± 254	-626 ± 247***	-352 ± 358	-478 ± 331***
Vol 3/Cell	μ^3	-40 ± 116	-560 ± 267***	-369 ± 285**	-457 ± 283***
DW/Cell	pg	-27 ± 48	26 ± 16**	6 ± 15	15 ± 18**
AW/Cell	pg	-2.4 ± 6.0	7.3 ± 6.0*	3.5 ± 3.2*	5.3 ± 4.9***
N/Cell	pg	-3.4 ± 4.9	4.9 ± 2.6***	1.4 ± 2.6	3.0 ± 3.1***
FAME/Cell	pg	-2.9 ± 5.2	12.7 ± 3.2***	4.0 ± 2.8***	8.0 ± 5.4***
TOM/Cell	pg	-24.9 ± 42.3	19.0 ± 11.8**	2.2 ± 12.4	10.0 ± 14.5*
NFOM/Cell	pg	-22.0 ± 37.1	6.2 ± 11.6	-1.8 ± 10.1	2.0 ± 11.1

*Significant at $p < .05$; **Significant at $p < .01$; ***Significant at $p < .005$

Table X. Means, standard deviations and percentage alterations of cell characteristics for six populations and nine later populations poisoned with 80 mg/1 KPCP. Percentage alterations from control values of cell contents due to poisoning with 40 mg/1 KPCP and 80 mg/1 KPCP.

	Units	Early Experiments Poisoned 80 mg/1 KPCP N = 6	Later Experiments Poisoned 80 mg/1 KPCP N = 9	Percent Change 40 mg/1 KPCP N = 6	Percent Change 80 mg/1 KPCP N = 13
Vol 1/Cell	μ^3	2176 ± 264	2046 ± 297	-2 %	17 %***
Vol 2/Cell	μ^3	1024 ± 428	1186 ± 636	-2 %	-30 %***
Vol 3/Cell	μ^3	585 ± 131	648 ± 156	-4 %	-42 %***
DW/Cell	pg	201 ± 26	194 ± 17	-12 %	8 %**
AW/Cell	pg	28.2 ± 8.9	25.8 ± 3.0	-9 %	25 %***
N/Cell	pg	30.5 ± 3.8	29.4 ± 2.8	-11 %	11 %***
FAME/Cell	pg	32.0 ± 3.3	24.4 ± 1.8	-13 %	41 %***
TOM/Cell	pg	173 ± 33	168 ± 14	-13 %	6 %*
NFOM/Cell	pg	139 ± 20	143 ± 15	-14 %	1 %

*Significant at $p<.05$; **Significant at $p<.01$; ***Significant at $p<.005$

Control Cells

On the tenth day after passage to fresh medium (Table II), the 144,000,000 cells had increased to 339,000,000 cells. They occupied a volume of 614 mm^3, had a dry weight of 64 mg, contained 8 mg of ash and 9 mg of nitrogen, and yielded 7 mg of fatty acid methyl esters (FAME). Dividing this population volume by the number of cells, 1873 cubic microns is obtained as the volume per cell (Table VIII). (This implies a cell diameter near 15 microns for a spherical cell.) Clearly the cell volume is much more than the 90 cubic microns of the human red blood cell.

The average volume of the control cells was 1873 cubic microns. At a density of 2.2 the 23 picograms of salts would occupy a volume of ten cubic microns; the 21 picograms of fats at a density of 0.7 would occupy 30 cubic microns; and the 145 picograms of non-fatty acid organic matter (NFOM) at a density of 1.3 would occupy 112 cubic microns (Table VIII). This leaves 1873 -10-30-112 or 1721 cubic microns occupied by water; at 23° C this volume of water would weigh 1717 picograms. Thus the cells contained the fraction 1717/(1717+194), 1717/1911, or approximately 90 percent water on a weight/weight basis and 92 percent (1721/1873) water on a volume/volume basis. On the basis of a fat-free cell the water content was 91 percent weight/weight.

Poisoned Cells

Data on cells poisoned with 40 mg KPCP/1 are given in the last column of Table VIII and data from their paired controls are given in the next to last column. The mean differences between control and poisoned cells and the standard deviations of the mean differences for parameters of the average cell are given in column one of Table IX. The average volume per cell, the average dry matter, ash or nitrogen per cell, and the average FAME, TOM and NFOM per cell in poisoned cells were not significantly different from these parameters in control cells. The water content of the cells poisoned with 40 mg KPCP/1 was calculated as 1791 cubic microns and this was not statistically different from 1721 cubic microns for control cells. The percentage of water in the poisoned cells was 1787/1979 or 90 percent on a weight/weight basis and 1791/1965 or 91 percent on a volume/volume basis. On the fat-free basis the water content was 91 percent weight/weight. Again there were no significant differences between controls and cells poisoned with 40 mg KPCP/1.

Data on the average cell content of six early experiments and nine later experiments with cells exposed to 80 mg KPCP/1 are tabulated in Tables VIII, IX, and X. The differences, with significance levels indicated, are contained in Table IX and percentage changes

with poisoning are given in Table X. Cells poisoned with 80 mg KPCP/l had a volume of 2098 cubic microns, 17 percent greater than control cells when estimated from determinations after one hour of centrifugation. The longer time period between cell divisions may help to explain the increased cell volume. After two and three hours of centrifugation, however, the volume of cells poisoned with 80 mg KPCP/l was 28 and 39 percent lower than corresponding control cell volume (Table VIII). This fact suggests an increased fragility of the cell membrane.

Cell volume (one hour centrifugation), dry matter, ash, nitrogen, FAME, and TOM (Table IX) per cell were statistically, significantly greater in the cells poisoned by 80 mg KPCP/l than they were in corresponding control cells. Only the non-fatty acid organic matter per cell was not significantly different from that of control cells.

The water content was calculated as 1949 cubic microns per poisoned cell. The water content was 1945/2142 or 91 percent on a weight/weight basis and 1949/2098 or 93 percent on a volume/volume basis. Although the data were available, it was not deemed worthwhile to evaluate the parameters discussed in this paragraph for every population studied. By inference calculated water contents and water percentages in cells poisoned with 80 mg KPCP/l differed significantly from controls.

Composition of Populations, Cells and Media after Ten Days Growth of Cultures of Embryonic Cells from Oncorhyncus kisutch

Characteristics of the population of cells cultured for ten days between transfers have been discussed as have the average characteristics of individual cells in the populations. Composition, concentrations and relative concentrations can be thought of as characterizing either the populations, the cells, or both. Data, expressed as mg/ml, on the composition or concentration in cells (or populations) after ten days growth, are given in Tables XI, XII and XIII; data expressed as mg/mg (relative concentrations) are given in Tables XIV, XV and XVI. In general each item tells its own story and control and poisoned populations can be easily compared.

In populations poisoned with 40 mg KPCP/1, the relative concentrations expressed as mg/mg were not significantly different from controls at the $p<.05$ level (Table XV). But the concentrations, expressed as mg of substance per ml of population volume (Table XII), differed from those of control populations at the 5 percent level of probability.

Most concentrations (Tables XII and XV) in populations poisoned with 80 mg KPCP/1 differed from controls at the $p<.001$ level. The ratio of nitrogen to dry weight differed only at the $p<.05$ level, and ash and nitrogen per ml of population volume did not differ from

Table XI. Means and standard deviations of cell component concentrations for control populations, populations poisoned with 80 mg/l KPCP and populations poisoned with 40 mg/l KPCP and their paired controls.

	Units	Control Populations N = 19	Poisoned Populations 80 mg/l KPCP N = 15	Control Populations N = 6	Poisoned Populations 40 mg/l KPCP N = 6
Vol 2/Vol 1	%	83 ± 14	52 ± 20	71 ± 20	71 ± 16
Vol 3/Vol 1	%	55 ± 12	29 ± 4	46 ± 9	45 ± 8
DW/Vol 1	mg/ml	105 ± 12	95 ± 8	110 ± 14	98 ± 5
AW/Vol 1	mg/ml	12.5 ± 1.3	12.8 ± 2.2	13.3 ± 1.1	12.4 ± 0.8
N/Vol 1	mg/ml	15.5 ± 1.6	14.4 ± 1.5	16.1 ± 1.7	14.6 ± 0.7
FAME/Vol 1	mg/ml	11.4 ± 1.6	13.2 ± 2.4	11.4 ± 1.6	10.2 ± 0.8
TOM/Vol 1	mg/ml	92.5 ± 11.0	81.8 ± 7.2	97.1 ± 12.6	85.9 ± 4.3
NFOM/Vol 1	mg/ml	80.2 ± 9.0	68.0 ± 6.1	84.6 ± 10.3	75.6 ± 3.8

Table XII. Mean differences from paired controls and standard deviations of the differences of concentrations of cell components for populations poisoned with 40 mg/l KPCP and 80 mg/l KPCP.

	Units	Poisoned-Control 40 mg/l KPCP N = 6	Poisoned-Control 80 mg/l KPCP N = 6	Poisoned-Control 80 mg/l KPCP N = 7	Poisoned-Control 80 mg/l KPCP N = 13
Vol 2/Vol 1	%	0 ± 6	-45 ± 15***	-30 ± 21***	-37 ± 19***
Vol 3/Vol 1	%	-1 ± 5	-38 ± 17***	-24 ± 5***	-30 ± 14***
DW/Vol 1	mg/ml	-12.1 ± 9.4*	-4.3 ± 5.2*	-11.4 ± 13.4*	-8.1 ± 10.7**
AW/Vol 1	mg/ml	-0.9 ± 0.7*	1.3 ± 2.1	0.2 ± 0.6	0.7 ± 1.6
N/Vol 1	mg/ml	-1.5 ± 1.2*	-0.2 ± 0.6	-1.4 ± 2.4	-0.9 ± 1.8
FAME/Vol 1	mg/ml	-1.3 ± 1.1*	4.2 ± 1.8***	0.4 ± 1.5	2.2 ± 2.5***
TOM/Vol 1	mg/ml	-11.2 ± 8.9*	-5.6 ± 4.6*	-11.6 ± 13.2*	-8.8 ± 10.2***
NFOM/Vol 1	mg/ml	-9.9 ± 7.9*	-9.8 ± 5.0***	-12.0 ± 11.8*	-11.0 ± 9.0***

*Significant at $p < .05$; **Significant at $p < .01$; ***Significant at $p < .005$

Table XIII. Means, standard deviations and percentage alterations of concentrations for populations poisoned with 80 mg/l KPCP. Percentage alterations from paired control values due to poisoning with 40 mg/l KPCP and 80 mg/l KPCP.

	Units	Early Experiments 80 mg/l KPCP N = 6	Later Experiments 80 mg/l KPCP N = 9	Percent Change 40 mg/l KPCP N = 6	Percent Change 80 mg/l KPCP N = 13
Vol 2/Vol 1	%	45.9 ± 14.9	55.8 ± 23.3	1 %	-58%***
Vol 3/Vol 1	%	26.6 ± 2.9	31.4 ± 4.3	-2 %	-50%***
DW/Vol 1	mg/ml	92.5 ± 5.4	95.9 ± 9.6	-11 %*	-8%**
AW/Vol 1	mg/ml	12.8 ± 0.5	12.7 ± 1.2	-7 %*	6 %
N/Vol 1	mg/ml	14.0 ± 3.3	14.7 ± 1.9	-9 %*	-6 %
FAME/Vol 1	mg/ml	14.9 ± 2.4	12.1 ± 1.7	-11 %*	20 %***
TOM/Vol 1	mg/ml	79.7 ± 3.6	83.2 ± 8.7	-12 %*	-10 %***
NFOM/Vol 1	mg/ml	64.7 ± 3.1	70.1 ± 7.8	-12 %*	-14 %***

*Significant at $p < .05$; **Significant at $p < .01$; ***Significant at $p < .005$

Table XIV. Means and standard deviations of ratios of cell components for control populations, populations poisoned with 80 mg/l KPCP and populations poisoned with 40 mg/l KPCP and their paired controls.

	Units	Control Populations N = 19	Poisoned Populations 80 mg/l KPCP N = 15	Control Populations N = 6	Poisoned Populations 40 mg/l KPCP N = 6
AW/DW	mg/mg	0.120 ± 0.011	0.135 ± 0.019	0.121 ± 0.007	0.127 ± 0.006
N/DW	mg/mg	0.148 ± 0.006	0.152 ± 0.005	0.146 ± 0.009	0.149 ± 0.002
FAME/DW	mg/mg	0.108 ± 0.008	0.140 ± 0.025	0.104 ± 0.004	0.103 ± 0.005
NFOM/DW	mg/mg	0.772 ± 0.010	0.724 ± 0.024	0.775 ± 0.007	0.770 ± 0.010
FAME/N	mg/mg	0.733 ± 0.061	0.925 ± 0.168	0.708 ± 0.045	0.694 ± 0.036
FAME/TOM	mg/mg	0.122 ± 0.008	0.158 ± 0.024	0.118 ± 0.004	0.118 ± 0.006
N/TOM	mg/mg	0.168 ± 0.008	0.175 ± 0.006	0.166 ± 0.011	0.170 ± 0.003
N/NFOM	mg/mg	0.192 ± 0.009	0.209 ± 0.009	0.189 ± 0.012	0.193 ± 0.004
FAME/NFOM	mg/mg	0.140 ± 0.012	0.194 ± 0.040	0.134 ± 0.005	0.134 ± 0.008

Table XV. Mean differences and standard deviations of cell component ratios for populations poisoned with 40 mg/1 KPCP and their paired controls, cell populations poisoned with 80 mg/1 KPCP and their paired controls.

	Units	Poisoned-Control 40 mg/1 KPCP N = 6	Early Experiments Poisoned-Control 80 mg/1 KPCP N = 6	Later Experiments Poisoned-Control 80 mg/1 KPCP N = 7	Combined Poisoned-Control 80 mg/1 KPCP N = 13
AW/DW	mg/mg	0.005 ± 0.007	0.019 ± 0.019*	0.015 ± 0.013*	0.017 ± 0.015***
N/DW	mg/mg	0.002 ± 0.008	0.005 ± 0.007	0.003 ± 0.003*	0.003 ± 0.006*
FAME/DW	mg/mg	0.000 ± 0.004	0.051 ± 0.023***	0.018 ± 0.009***	0.033 ± 0.024***
NFOM/DW	mg/mg	-0.005 ± 0.009	-0.070 ± 0.019***	-0.033 ± 0.018***	-0.050 ± 0.026***
FAME/N	mg/mg	-0.014 ± 0.048	0.311 ± 0.164***	0.104 ± 0.060***	0.200 ± 0.156***
N/TOM	mg/mg	0.004 ± 0.010	0.009 ± 0.009*	0.006 ± 0.003***	0.007 ± 0.007***
FAME/TOM	mg/mg	0.000 ± 0.005	0.061 ± 0.024***	0.023 ± 0.010***	0.040 ± 0.026***
N/NFOM	mg/mg	0.004 ± 0.012	0.025 ± 0.010***	0.012 ± 0.006***	0.018 ± 0.010***
FAME/NFOM	mg/mg	0.005 ± 0.063	0.087 ± 0.037***	0.030 ± 0.014***	0.056 ± 0.039***

*Significant at $p < .05$; **Significant at $p < .01$; ***Significant at $p < .005$

Table XVI. Means, standard deviations and percentage alterations of cell component ratios for populations poisoned with 80 mg/1 KPCP. Percentage change from paired control values due to poisoning with 40 mg/1 KPCP and 80 mg/1 KPCP.

	Units	Early Experiments Poisoned 80 mg/1 KPCP N = 6	Later Experiments Poisoned 80 mg/1 KPCP N = 9	Percent Change 40 mg/1 KPCP N = 6	Percent Change 80 mg/1 KPCP N = 13
AW/DW	mg/mg	0.138 ± 0.029	0.133 ± 0.008	4 %	14 %***
N/DW	mg/mg	0.152 ± 0.005	0.152 ± 0.005	1 %	2 %*
FAME/DW	mg/mg	0.161 ± 0.027	0.126 ± 0.011	0 %	31 %***
NFOM/DW	mg/mg	0.701 ± 0.013	0.737 ± 0.010	-1 %	-6 %***
FAME/N	mg/mg	1.062 ± 0.176	0.834 ± 0.077	-2 %	28 %***
N/TOM	mg/mg	0.176 ± 0.005	0.175 ± 0.006	2 %	4 %***
FAME/TOM	mg/mg	0.184 ± 0.023	0.145 ± 0.012	0 %	34 %***
N/NFOM	mg/mg	0.216 ± 0.004	0.124 ± 0.003	2 %	10 %***
FAME/NFOM	mg/mg	0.230 ± 0.040	0.174 ± 0.017	3 %	41 %***

*Significant at $p<.05$; ***Significant at $p<.005$

controls.

Variability of Parameters

During the course of development of the data some unexpected groupings of the parameters with respect to variability were noted. The parameters are tabulated in Tables XVII A, B and C in order of percentage variability, with the parameters with greatest variation at the top and those of less or little variation at the bottom of the page. Table XVII A lists variabilities noted in the control experiments, expressed as a coefficient of variation, and computed as the ratio of the standard deviation to the mean. For an average of six experiments a displacement of the mean by one standard deviation can be expected by chance about once in 40 times. This displacement in some respects is equivalent to the displacement of control means induced by 40 mg KPCP/l. Tables XVII B and XVII C give the percentage deviations from controls induced by 40 mg and 80 mg KPCP/l. An attempt has been made to keep positions (from top to bottom) of similar variability constant in Tables XVII A, B and C.

The most variable items in control experiments were the population volumes after three hours (32 percent) and after two hours (25 percent) of centrifugation. Thus, most variable was the susceptibility to damage on prolonged centrifugation. Cell numbers had an 18 percent population volume, a 14 percent population ash, a 13

Table XVII A. Order of variability: parameters from control cultures expressed as standard deviation/mean.

Population Parameters		Cell Parameters		Concentrations mg/ml of Population		Complex Concentrations Relationships	
3 hr Volume	.32						
2 hr Volume	.25	Ash/cell	.26				
		Volume/cell	.24				
		DW/cell	.23				
		FAME/cell	.22				
		N/cell	.21			Ash/FAME	.17
Cell Number	.18	NFOM/cell	.18				
1 hr Volume	.14			[FAME]	.14		
Ash	.13			[TOM]	.12		
				[Dry Weight]	.11		
				[NFOM]	.11		
				[Ash]	.11	Ash/N	.11
FAME	.10			[Nitrogen]	.10	Ash/NFOM	.10
						Ash/DW	.09
						FAME/NFOM	.08
						FAME/N	.08
NFOM	.07					FAME/DW	.08
TOM	.07					FAME/TOM	.07
DW	.07						
N	.07						
						N/TOM	.05
						N/NFOM	.05
						N/DW	.04
						NFOM/DW	.01

Table XVIIB. Order of variability: displacement of parameter means in cultures poisoned with 40 mg KPCP/l.

Population Parameters		Cell Parameters		Concentrations mg/ml of Population		Complex Concentrations Relationships	
						$NFOM/H_2O$	.16
						$FAME/H_2O$	.15
		NFOM/cell	-.14				
		FAME/cell	-.13				
H_2O	.12	DW/cell	-.12	[TOM]	-.12*		
				[NFOM]	-.12*		
		N/cell	-.11	[DW]	-.11*	Ash/H_2O	.11
				[FAME]	-.11*		
		Ash/cell	-.09	[Nitrogen]	-.09*		
				[Ash]	-.07*		
Cell Count	.06						
2 hr Volume	.06						
TOM	-.06***						
1 hr Volume	.05					Ash/FAME	.05
DW	-.05**					Ash/NFOM	.05
FAME	-.05*						
NFOM	-.05***						
Nitrogen	-.04					Ash/DW	.04
						N/H_2O	.04
3 hr Volume	.03						
						FAME/NFOM	.03
						Ash/N	.03
						N/NFOM	.02
		Volume/cell	-.02			FAME/N	-.02
Ash	-.01	H_2O/cell	.01			N/DW	.01
						FAME/DW	.00
						N/H_2O	.00

*** Statistically significant at $p < 0.001$

** Statistically significant at $p < 0.01$

* Statistically significant at $p < 0.05$

Items not starred were not significantly different from controls.

Table XVII C. Order of variability: displacement of parameter means in cultures poisoned with 80 mg KPCP/l.

Population Parameters		Cell Parameters		Concentrations mg/ml of Population		Complex Concentrations Relationships	
3 hr Volume	-.66						
2 hr Volume	-.59						
Cell Count	-.43						
NFOM	-.41	FAME/cell	.41			FAME/NFOM	.41
TOM	-.39						
Dry Weight	-.38						
Nitrogen	-.36						
1 hr Volume	-.33					FAME/TOM	.34
						FAME/DW	.31
Ash	-.29					FAME/N	.28
H_2O	-.29						
		Ash/cell	.25				
FAME	-.20			[FAME]	.20		
		Volume/cell	.17			Ash/NFOM	.18
				[NFOM]	-.14	Ash/DW	.14
		H_2O/cell	.13			FAME/H_2O	.14
						Ash/H_2O	.12
		N/cell	.11			Ash/N	.11
				[TOM]	.10	N/NFOM	.10
		DW/cell	.08**	[Dry Weight]	.08**		
				[Ash]	.06°	NFOM/H_2O	.06
				[Nitrogen]	-.06°		
						NFOM/DW	-.06
						N/TOM	.04
						Ash/FAME	.03°
						N/DW	.02*
		NFOM/cell	.01°				
						N/H_2O	.00°

Most items were statistically significant at $p < 0.001$. Items not statistically significant are indicated by the symbol °; items significant at $p < 0.05$ and $p < 0.01$ are indicated by * and **.

percent, and population fatty acids a 10 percent coefficient of variability. Population NFOM, TOM, dry weight and nitrogen had 7 percent coefficients of variability (Table XVII A).

The average volume, ash, dry weight, FAME, nitrogen and non-fatty acid organic matter per cell had coefficients of variability between 0.26 and 0.18 and except for cell number were well outside the range of 0.14 to 0.07 obtained for the population parameters.

The coefficients of concentrations of FAME, TOM, dry weight, NFOM, ash and nitrogen ranged from 0.14 to 0.10, were similar to coefficients of ash and FAME in the populations, were well above the value of 0.07 noted for other population parameters, and were well below the coefficients for cell parameters. The complex concentration relationships had low coefficients between 0.11 and 0.01. The ratios N/TOM, N/NFOM, N/DW and NFOM/DW had coefficients of 0.05 or lower.

For control cultures variability decreased sequentially from cell parameters to population number and population volume, to concentrations per ml, to population weight parameters, to interrelations between ash, FAME, NFOM and nitrogen. This sequence was mildly disturbed by 40 mg KPCP/l (population number and volume) and was disrupted by 80 mg KPCP/l.

	Controls	40 mg KPCP/1	80 mg KPCP/1
Cell weight parameters	0.18-0.26	0.09-0.14	0.01-0.41
Population number and volume	0.14-0.18	0.05-0.06	0.33-0.43
Concentrations per ml	0.10-0.14	0.07-0.12	0.06-0.20
Population weight parameters	0.07-0.13	0.01-0.06	0.20-0.41
Interrelations: Ash, FAME, NFOM, N	0.01-0.17	0.01-0.05	0.02-0.41

The displacement of the means of all population parameters by 40 mg KPCP/1 was considerably less than indicated by the coefficients of variation in controls. It is worth commenting that ash per cell and cell volume had the greatest coefficients of variation in controls but the means of ash and volume were displaced less by 40 mg KPCP/1 than were other cell parameters. The greatest displacement was 0.14 for NFOM/cell. The averages of material concentrations for cells and populations were displaced slightly less than one standard deviation, but were sufficiently displaced to yield statistically significant differences.

Although FAME/N, Ash/NFOM, and N/NFOM had variability coefficients of 0.08, 0.10 and 0.05, the percentage displacement of these means induced by 40 mg KPCP/1 poisoning was only 0.02, 0.05 and 0.02, a fact indicating a high correlation between nitrogen, and FAME and NFOM, and between ash and NFOM in paired samples.

The highest coefficient of variability in controls was 0.26. Fifteen out of 40 of percentage displacements of means by 80 mg KPCP/l were above 0.26 and only 11 of the 40 were below 0.10. Population parameters and parameters involving fatty acids were extensively disturbed; and roughly, cell parameters, concentrations and relative concentrations were similarly disturbed. However, the range was large and some items of each group were definitely displaced and other items of each group were stable.

In controls, the concentration of nitrogen, and the relative concentrations expressed by N/NFOM and N/DW had low variability indices (0.01-0.10), and the percentage displacements of the means induced by 40 and 80 mg KPCP/l were low.

Alterations in Media During Growth

The volumes of the media were about 260 times the volumes of the cell populations after ten days growth. Hence, the compositions of the media were not altered markedly in many respects by the growing cells (Table XVIII, middle). Water concentrations of the media did not change. The ash concentrations of the media were slightly reduced by 40 mg KPCP/l. NFOM concentrations of the media were slightly reduced during growth. Fatty acid concentrations were reduced from 103 to 60 mg per liter in control cultures and cultures treated with 40 mg KPCP/l, and to 75 mg per liter

Table XVIII. Concentrations of water, ash, FAME and NFOM in cells and media.

		Control		40 mg KPCP/1	80 mg KPCP/1
		Day 0	Day 10	Day 10	Day 10
Cells					
Water	mg/ml	896	896	903	906
Ash	mg/ml	12.9	12.9	12.4	12.8
FAME	mg/ml	11.4	11.4	10.2	10.2
NFOM	mg/ml	82.3	82.3	75.6	68.0
Media					
Water	mg/ml	983	983	983	983
Ash	mg/ml	9.39	9.41	9.35	9.42
FAME	mg/ml	0.103	0.063	0.060	0.075
NFOM	mg/ml	13.94	13.88	13.84	13.88
Cells/Media					
Water	mg/ml		.91	.919	.92
Ash	mg/ml		1.37	1.33	1.36
NFOM	mg/ml		5.9	5.5	4.9
FAME	mg/ml		181	170	136

in cultures poisoned with 80 mg KPCP/1.

Cells had lower water concentrations than the media, but higher ash, FAME and NFOM concentrations. Ash in the cells was concentrated to a level 33-37 percent above the ash concentration of the media. NFOM was concentrated to a level five to six times, and FAME in controls to a level 180 times, and in poisoned cultures to 136-170 times the level in the media.

Identification of Fatty Acids in Cell Populations

Fatty acid methyl esters were prepared from extracted, saponified lipids to identify the individual fatty acids present in cell populations. The FAME were detected by gas-liquid chromatography (GLC). Comparisons of chromatographic records of individual fatty acid methyl esters of known purity obtained from the Hormel Institute (and the chromatograms obtained by coincident injection of Hormel standard samples with unknown cell mixtures) with the chromatograms of cell population FAME identified some of the cell fatty acids. The fatty acids from the Hormel Institute were 8:0, 10:0, 12:0, 14:0, 16:0, 18:0, 20:0, 16:1 ω7, 18:1 ω9, 18:2 ω6, 20:4 ω6, 20:5 ω3 and 22:6 ω3.

Fatty acids remained in the chromatograph column from a few seconds to two and one-half hours depending upon their physical characteristics. Fatty acids with N carbon atoms came off before

acids with N + 1 carbons; acids of the same number of carbons with N double bonds came off before acids with N + 1 double bonds; and for acids of the same number of carbons and double bonds, those with double bonds located more toward the interior of the molecule came off slightly earlier. Thus, if the positions of fatty acids on chromatorgrams were known in comparison with Hormel standards, a reasonably accurate identification of all fatty acid peaks could be made by simple inspection. A straight line was obtained by plotting the logs of the retention times of individual Hormel saturated fatty acid methyl esters against the number of carbons in their respective chains. Another parallel straight line was drawn through the log retention time vs carbon number points of 16:1 and 18:1. When fatty acid methyl ester retention times were plotted, a verification or negation of the carbon numbers assigned by inspection could readily be obtained.

Separation of the cell FAME mixture by silver nitrate thin-layer plates into fractions of esters with the same numbers of double bonds allowed the production of GLC chromatograms with peaks for saturated fatty acids or for unsaturated fatty acids with the same number of double bonds. Hydrogenation of these separated groups allowed precise determination of the number of carbons for the unsaturated fatty acids and confirmation of the saturation of saturated fatty acids.

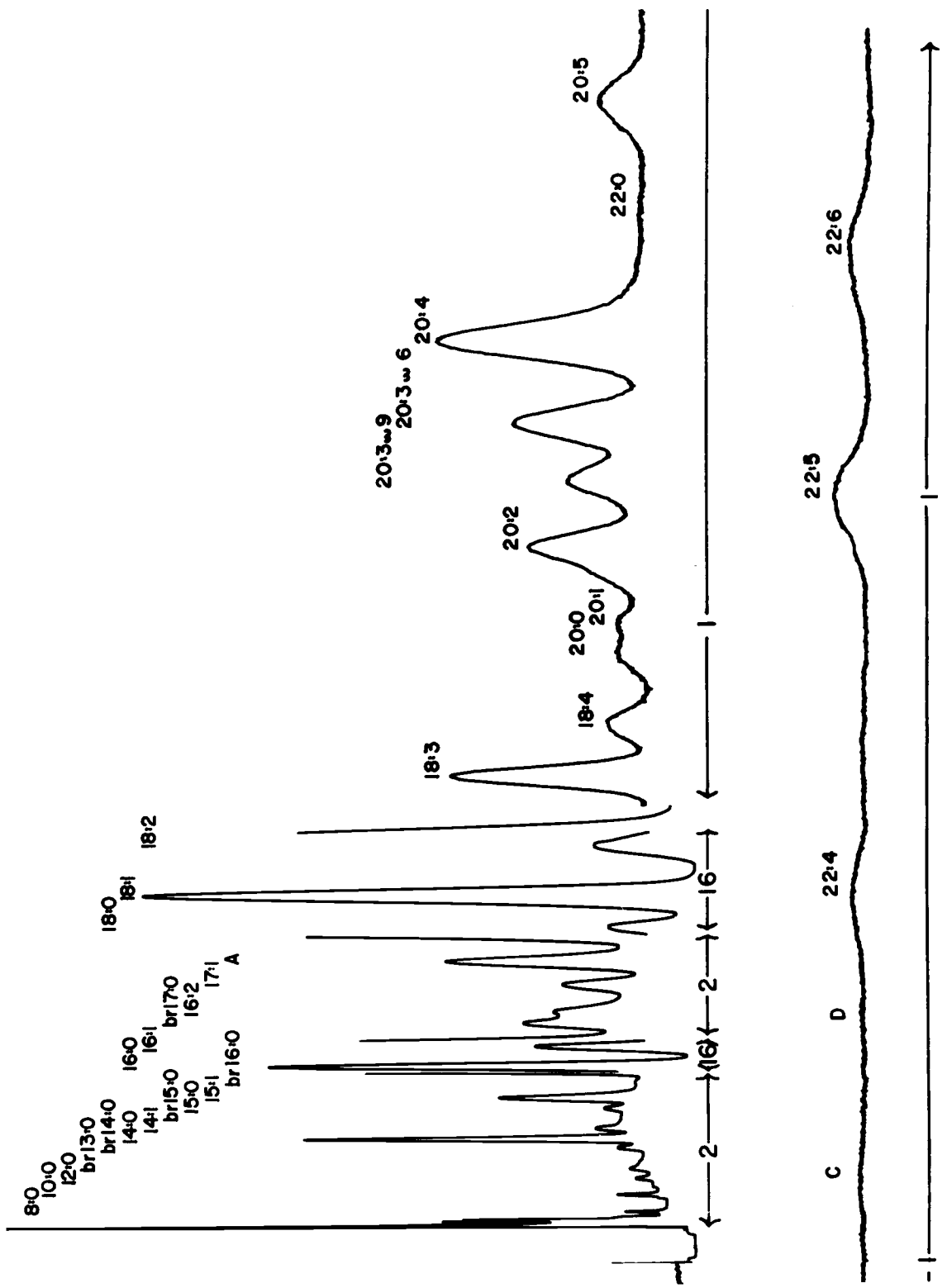
Ozonolysis of the unsaturated fractions separated by thin-layer chromatography and subsequent comparisons of gas chromatograms of the ozonolytic products with the chromatograms of products from Hormel standard acids aided in the determination of the position of some of the double bonds in the unsaturated acids. A determination of the separation factors, modified from Ackman (1, 3), between the time of emergence of unsaturated acids from the gas chromatographic column and the time of emergence of the saturated acid of the same number of carbon atoms also aided in determining the double bond position of unsaturated acids.

A sample chromatogram showing detector response to the fatty acid methyl ester mixture obtained from control cells is given as Figure 2. Individual fatty acids are numbered at the top with the number of each beginning directly above its peak maximum. The line and numbers at the bottom of the figure designate the factor by which the voltage response to the recorder was diminished to reduce the height of peaks on the chromatogram.

Saturated Fatty Acids

The thin-layer fraction of fatty acids from the cell FAME mixture which was nearest the solvent front developed 12 peaks on a gas chromatogram, none of which changed position when the sample was hydrogenated. Some minor and all major peaks matched peaks

Figure 2. Sample chromatogram of control population fatty acid methyl esters. The ordinate represents detector response, the abscissa time. Total time was approximately three hours. The figure before the colon gives the number of carbons and that after the colon the number of double bonds. The carbon number is placed directly above the peak maximums. Numbers below the peaks indicate the factors by which reorder response was reduced to keep large peaks on the chart.


8:0
10:0
12:0
br13:0
br14:0
14:0
14:1
br15:0
15:0
15:1
br16:0
16:0
16:1
br17:0
16:2
17:1
A
18:0
18:1
18:2
18:3
18:4
20:0
20:1
20:2
20:3ω9
20:3ω6
20:4
20:5
22:0
22:3
22:4
22:6
C
D
2
(16)
2
16
1
1

made by Hormel's standard fatty acid methyl esters upon concomitant injection of the two samples.

These matching peaks and some others for which Hormel standards were not available plotted as a straight line (log retention time vs carbon number). The peaks were identified as those of saturated fatty acid methyl esters 8:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0 and 22:0. Other minor peaks immediately preceded the emergence of saturated acids from the gas chromatographic column, and plotted on a line parallel to the plotted line of the above acids. These peaks were assumed to be branched chain, saturated fatty acids and designated br13:0, br14:0, br15:0, br16:0 and br17:0.

Unsaturated Fatty Acids

Fatty acid methyl esters separated into distinct bands on silver nitrate, thin-layer plates according to the number of double bonds in the molecule, with the saturated acids displaced the most from the origin and the unsaturated acids displaced less as the number of double bonds increased (31). Comparison of the chromatographic peaks obtained from Hormel standards 16:1, 18:1, 18:2, 18:3, 20:4, 20:5, and 22:6 with the chromatographic peaks of cell FAME gave direct, preliminary identification of these

unsaturated fatty acids.

Tentative assignation of chain length and number of double bonds for the remaining unsaturated fatty acids were derived from the position of the peaks with reference to identified peaks. The semilog plot of the log retention time against assigned numbers of carbons gave a series of parallel straight lines for acids with no, one, two, three, four and five double bonds. The line for N + 1 double bonds lay above and parallel to the line for N double bonds.

Cell Fatty Acids Containing One Double Bond

The fatty acids of cell populations which were identified as containing one double bond were 14:1, 15:1, 16:1 ω7, 17:1, 18:1 ω9, 19:1, 20:1 ω9 and a 16 carbon acid with one carbon-carbon triple bond. Comparison with retention times of Hormel standards and concomitant injection of the two samples gave direct identification of fatty acids 16:1 and 18:1. The other fatty acids containing one double bond were tentatively identified on the basis of their chromatographic positions relative to the known fatty acids and by semilog plotting.

Further identification of the fatty acids containing one double bond was obtained by separation of the FAME on silver nitrate, thin-layer plates. GLC analysis of the fraction containing the monene fatty acids revealed eight peaks that verified the carbon numbers originally assigned and established the efficacy of the separation.

Hydrogenation reduced the number of peaks to seven and the individual retention times were changed to the retention times of the corresponding saturated acids. Thus a comparison between the original chromatogram, the chromatogram containing the FAME with one double bond, and the chromatogram after saturation, provided convincing evidence for the number of carbons in the chains of the monene fatty acids. One peak, which accounted for somewhat over two percent of the original sample, did not plot on the straight line formed by the other monene acids from either the chromatogram of the original FAME mixture or that of the silver nitrate thin-layer separated fraction. Most of this fatty acid consistently occurred in the monene thin-layer fraction although a significant amount also occurred in the diene fraction. Upon hydrogenation it apparently was reduced to the 16 carbon saturated acid. Ozonolytic products were too minor in quantity to be conclusive. The retention time of this acid in the gas chromatograph relative to acid 18:0 was between 0.88 and 0.90. On these bases this acid was designated as a fatty acid with 16 carbons and one triple bond, possibly in the ω9 position. Two papers (33, 42) indicate that a monyne acid has thin-layer and gas chromatographic properties similar to those observed here. However, because the identification of this acid does not rest on as strong evidence as the identification of other acids, this acid is termed "A" in subsequent discussions.

The positions of the double bonds in acids 16:1 ω7 and 18:1 ω9 were confirmed by gas-chromatographic analysis of the ozonolytic products formed from the monene fraction of FAME. The minute quantities involved and microcontaminants from the procedures involved did not allow any degree of certainty in assigning double bond position in the other monenes. A modification of Ackman's separation factors (1, 3) however allows assignation of these positions by accurately plotting the acid's gas chromatographic retention time and comparing it to the retention time of its saturated counterpart. The double bond position is a linear function of the time separation between unsaturated acids and their saturated counterparts vs number of double bonds for any acids of the same number of carbons. The position of the double bond in acid 20:1 can be assumed to be in the ω9 position.

Cell Fatty Acids Containing Two Double Bonds

The fatty acids of cells which were identified as containing two double bonds were 16:2 ω7, 17:2, 18:2 ω9 and 20:2 ω9. Comparison with retention time of Hormel standard acid 18:2 ω6 and concomitant injection of this standard with the FAME extracted from cells established the basis for carbon number and semilog plotting of the unknown sample acids. Since the standard acid was an ω6 acid, however, its peak did not match the 18:2 ω9 peak from the sample.

Upon concomitant sample injection, the 18:2 peak was broadened. The other fatty acids containing two double bonds were tentatively identified on the basis of their chromatographic positions relative to known fatty acids and by semilog plotting.

The fatty acids containing two double bonds separated into the third band from the solvent front on silver nitrate thin-layer plates. GLC chromatograms of this fraction contained five peaks which reduced to four peaks upon hydrogenation. The four peaks of the hydrogenated sample matched those of known saturated fatty acid methyl esters. One of the peaks of the thin-layer fraction matched one discussed above and designated a triple bonded, 16 carbon acid. Gas chromatograms of the ozonolytic products of the thin-layer fraction revealed that aldehydes of seven and nine carbons and aldehyde-esters of six and eight carbon chains were present. Proportions of the aldehydes and aldehyde-esters present called for identification of the original acids as 16:2 $\omega 7\Delta 6$, 18:2 $\omega 9\Delta 6$ and 20:2 $\omega 9\Delta 8$. The small quantity of 17:2 present did not allow its positive identification because a standard, unsaturated, 17 carbon acid was not available. Deriving the position of the double bond by accurate plotting of the retention time was also impossible.

Cell Fatty Acids Containing Three Double Bonds

The fatty acids of cells which were identified as containing three double bonds were 18:3 ω3, 20:3 ω9 and 20:3 ω6. Semilog plotting allowed tentative identification of these acids along with inspection of retention times in the gas chromatograph and comparisons with known fatty acid methyl esters.

Silver nitrate thin-layer plates caused a separation of these acids into a band immediately above the origin. A gas-chromatogram of this fraction contained only three major peaks. When the sample was hydrogenated and reinjected into the chromatograph, the number of major peaks was reduced to two which coincided with the peaks of 18 and 20 carbon, saturated acids.

Chromatograms of the ozonolytic products of the fraction limited the subsequent assignment of double bond position, but was not conclusive due to a relatively high level of background contamination. Plotting the retention times accurately in a modification of Ackman's separation factors however, designated these acids as 18:3 ω3Δ9, 20:3 ω9Δ5 and 20:3 ω6Δ8.

Cell Fatty Acids Containg Four, Five and Six Double Bonds

The fatty acids of cells which were identified as containing four, five and six double bonds were 18:4 ω3, 20:4 ω6, 20:5 ω3,

22:4 ω6, 22:5 ω3 and 22:6 ω3. Acids 20:4 ω6, 20:5 ω3 and 22:6 ω3 were directly identified by coincident injection of the three Hormel Standard FAME and the cell sample. These standard acids also were the basis of carbon number assignments and semilog plotting of the retention times.

The origin of the silver nitrate thin-layer plate produced five major peaks when the fraction was injected into the gas chromatograph. All of these matched the above acids, but acid 22:6 was not present. Since the amounts of acids were small and the highly unsaturated acids are easily oxidized by exposure to air, the fraction was not replated to separate acids with different numbers of double bonds.

Hydrogenation of the thin-layer, separated fraction produced three major peaks on a gas chromatogram which corresponded to those of the saturated acids with 18, 20, and 22 carbons per molecule. The proportions of these acids which were formed helped to confirm tentative identification. Ozonolytic products of the thin-layer fraction confirmed the tentatively assigned positions of double bonds as 20:4 ω6Δ5, 20:5 ω3Δ5, 22:4 ω6Δ7, and 22:5 ω3Δ7. Plots of the retention times of acids 18:4 ω3 and 22:6 ω3 confirmed the position of the double bonds in these molecules.

Cell Fatty Acids of Unknown Structure

Four minor peaks which were sometimes resolved by the gas chromatograph could not be identified. The 16 carbon monyne bonded acid will also be discussed with this group since it is not well identified. These acids will be assigned letters and identified by their retention times relative to the retention time of stearic acid (18:0). They are acids A (16 carbon monyne), B, C, D and E with relative retentions of 0.87-0.90 (A), 3.2 (B), 4.1-4.3 (C), 4.5-4.9 (D) and 5.8-6.2 (E).

Summary

The fatty acids of the cell populations were identified as:

8:0							
10:0							
12:0							
	br13:0						
14:0	br14:0	14:1					
15:0	br15:0	15:1					
16:0	br16:0	16:1 ω7	16:2 ω7				
17:0	br17:0	17:1	17:2				
18:0		18:1 ω9	18:2 ω9	18:3 ω3	18:4 ω3		
		19:1					
20:0		20:1 ω9	20:2 ω9	20:3 ω9	20:4 ω6	20:5 ω3	
				20:3 ω6			
22:0					22:4 ω6	22:5 ω3	22:6 ω3

Quantitative Estimation of Percentage Contributions of Individual Fatty Acids

Data of the percent contributions of individual FAME to the total FAME of a sample are useful as they reflect the relative concentrations of individual fatty acids in the sample. Differences in the percent contributions of individual fatty acids between day zero medium, control populations, poisoned populations and day ten media reflect processes which were selective for individual fatty acids.

Some acids are maintained in cells against concentration gradients while others may be excluded from cells against concentration gradients. If the percentage contribution of an acid is greater in both cells and day ten medium than in day zero medium, this acid was catabolized on a net basis less than average and it may have been synthesized. If the percentage contribution of an acid is greater in the cells and less in the depleted medium than that of day zero medium, this acid was selectively absorbed from the medium. If the percentage contribution of an acid is less in the cells and greater in the depleted medium than that of day zero medium, this acid may have been selected against in absorption by the cells and both synthesized and excreted by the cells against a concentration gradient.

Control Populations

Table XIX includes the mean percent compositions of individual fatty acid methyl esters for day zero medium, control cell populations and the day ten media depleted by the growth of control populations.

Saturated Fatty Acids

The saturated fatty acid content of day zero medium is very high (42.5 percent). Control populations selectively depleted the saturated fatty acid content of the medium in ten days to 32.2 percent of the total FAME while maintaining a relatively low percentage of saturated fatty acids in the cells (24.6 percent). These figures suggest that the saturated fatty acids are used either as a major source of energy by their catabolic oxidation or as precursors to the synthesis of other cell components.

The relative proportions of 20:0 and 22:0 (of minor content) are significantly increased in the medium after ten days of cell growth. Acid 20:0 is present in a higher percentage in the cells than in the media; this indicates selective net retention by the cells. Acid 22:0 comprises the same percentage of the fatty acids in the cells and in fresh medium.

Table XIX. Mean individual fatty acid percentages of total FAME in day 0 medium, day 10 control populations and their depleted media.

Fatty Acid	Relative Retention Time	Day 0 Medium N = 7	Control Populations N = 19	Control Media N = 13
8:0	0.04-0.05	0.00	0.01	0.00
10:0	0.09-0.10	0.01	0.02	0.01
12:0	0.15-0.17	0.13	0.02	0.04
14:0	0.27-0.30	1.15	0.98	1.03
15:0	0.38-0.41	0.22	0.21	0.29
16:0	0.52-0.54	25.85	15.22	17.60
17:0	0.72-0.74	0.51	0.23	0.60
18:0	1.00	14.51	7.38	12.24
20:0	1.8-2.0	0.01	0.48	0.08
22:0	3.3-3.5	0.10	0.06	0.28
br13:0	0.18-0.20	0.00	0.08	0.10
br14:0	0.24-0.27	0.04	0.37	0.54
br15:0	0.35-0.37	0.17	0.18	0.22
br16:0	0.47-0.49	0.12	0.08	0.12
br17:0	0.66-0.71	0.21	0.89	0.29
14:1	0.31-0.34	0.05	0.14	0.11
15:1	0.41-0.45	0.10	0.46	0.18
16:1ω7	0.58-0.62	2.77	7.09	4.02
17:1	0.79-0.84	0.43	0.89	0.78
18:1ω9	1.1	34.96	39.19	31.46
19:1	1.6	0.00	0.07	0.00
20:1ω9	2.0-2.1	0.20	0.20	0.44
16:2ω7	0.68-0.71	0.00	0.21	0.00
17:2	0.89-0.91	0.00	0.12	0.00
18:2ω9	1.2-1.4	11.42	10.07	19.64
20:2ω9	2.2-2.3	0.01	1.02	0.28
18:3ω3	1.5-1.6	0.15	1.66	0.62
20:3ω9	2.5-2.6	0.09	0.68	0.33
20:3ω6	2.7-2.8	1.22	2.12	1.38

Continued

Table XIX.

Fatty Acid	Relative Retention Time	Day 0 Medium N = 7	Control Populations N = 19	Control Media N = 13
18:4ω3	1.6-1.7	0.80	0.47	1.40
20:4ω6	2.9-3.0	2.75	3.89	3.22
22:4ω6	5.3-5.7	0.25	0.49	0.18
20:5ω3	3.7-3.9	0.54	1.11	0.74
22:5ω3	6.6-7.0	0.73	1.33	0.62
22:6ω3	7.4-7.8	0.46	0.83	0.34
A	0.87-0.90	0.04	1.54	0.10
B	3.2	0.00	0.05	0.06
C	4.1-4.3	0.07	0.09	0.16
D	4.5-4.9	0.00	0.03	0.12
E	5.8-6.2	0.03	0.05	0.17
Fatty Acid Mass		16,520μg	6,884μg	10,050μg

Branch Chain Fatty Acids

The fatty acids thought to have a branching chain comprise a small percentage of day zero medium (0.5 percent). Over the ten day growth period, control populations selectively augmented the proportion of branched chain acids in the medium to 1:3 percent of the total FAME while remaining at 2 percent of the FAME in the cells. Since the percentage composition of the branched acids in depleted medium and control populations was increased over that of day zero medium, a lower than average net catabolism and possible net synthesis of these acids by the cells took place. All of the individual branched acids seem to have been handled similarly.

Monene Fatty Acids

The FAME of day zero medium contained a large proportion of monene acids (38.5 percent) which was primarily acid 18:1. The depleted medium contained 37.0 percent monene acids after ten days of control population growth. The cellular FAME were 48.0 percent monene acids. Acid 18:1 was increased in the cell populations evidently at the expense of the medium. All other monene acids increased in percentage in depleted media. Both depleted media and cell populations had higher percentages of 18:1 than did fresh medium. This indicated a lower than average catabolism and possible net

synthesis of all monene acids with the exception of 18:1.

Diene Fatty Acids

Day zero medium FAME was 11.4 percent diene acids, the greater part being 18:2. The FAME of day ten depleted media contained 18.9 percent diene acids again almost entirely acid 18:2. The FAME of control populations were 11.4 percent dienes. Acids 16:2, 17:2 and 20:2 were synthesized and retained by the cells as there was little, if any, of these acids in the media. The FAME of the cell populations and day zero mediumwere composed of approximately equal percentages of acid 18:2 indicating essentially average net absorption, but the FAME of depleted medium had an increased percentage of 18:2 indicating possible synthesis and excretion by the cell population.

Triene Fatty Acids

The percentage of the triene fatty acids increased from 1.5 percent in day zero medium to 4.5 percent in control cell populations and 2.3 percent in the depleted media. The net catabolism of each of the three triene acids was less than average and net cellular retention was high.

Tetraene Fatty Acids

The relative percentages of fatty acids with four double bonds were not consistent. Tetraenes comprised 3.8 percent of the FAME from day zero medium, 4.8 percent from depleted media and 4.8 percent from control populations. Acid 18:4 had a lower percentage in cell populations, but higher in depleted media indicating poor relative net absorption from the medium. Acid 20:4 had a lower than average net catabolism. Acid 22:4 was absorbed and retained better by the cells than were most fatty acids.

Pentene and Hexene Fatty Acids

The two pentene acids (20:5 and 22:5) comprised 1.3 percent of the total day zero medium FAME, 2.4 percent of the total cell population FAME and 1.4 percent of the FAME in depleted media. These data suggest lower than average net catabolism or synthesis by the cell populations and poorer than average absorption from the medium. Acid 22:6 was concentrated to some extent in the cells, possibly by absorption from the medium.

Unknown Fatty Acids

All of the unidentified fatty acids comprised minute amounts of the total FAME from day zero medium. Day ten depleted media

contained a higher percentage of these acids as did the cell populations. These data suggest that the acids were synthesized by the cells with some minor loss to the media. Acid A, the possible monyne acid, was synthesized extensively as it comprised 1.5 percent of the total FAME from cells, but was in day zero medium in only trace amounts. Very little of this acid was lost to the medium after synthesis.

Summary

In general saturated fatty acids were readily absorbed from the media and catabolized or converted to other cell products. Branched chain and unsaturated acids tended to undergo less than average net catabolism by cell populations and were either poorly absorbed from the medium or lost again from the cells after synthesis. All of the monene acids made higher percentage contributions to the FAME of cell populations than to day zero medium. This could have been due to lower than average catabolism of these acids or possible synthesis. The increased percentage of acid 18:1 in cell populations was most likely due to a better than average absorption as the day ten media were depleted of acid 18:1. With few exceptions the polyunsaturated acids tended to have higher percentage contributions to the total FAME of cell populations than to day zero medium. Often their percentages were also higher in day ten media than

in day zero medium indicating that the polyunsaturated acids tended to be selectively conserved if not synthesized by cell populations.

KPCP Poisoned Populations

Table XX includes the mean individual fatty acid percentage contributions to total FAME in populations and depleted media from cell populations poisoned with 40 mg KPCP/1 and 80 mg KPCP/1.

Saturated Fatty Acids

Populations poisoned with 40 mg KPCP/1 reduced the percent contributions (to total fatty acids) of saturated acids in the medium from 42.5 percent to 34.0 percent while the level in the cells remained at 23.8 percent. Acids 15:0, 20:0 and 22:0 increased slightly in their relative percentage in the media. The relative percentages of acids 14:0, 17:0 and 18:0 remained rather stable while the percentage of acid 16:0 underwent a large drop in the media over the ten day growth period. The individual percentages of saturated fatty acids of the poisoned cell populations were lower than those of day zero medium with the exception of acid 20:0. Large drops in percent composition were experienced by acids 16:0, 17:0, and 18:0. These data indicate that poisoned cells in general catabolize saturated acids at a greater than average net rate using the fatty acids as metabolic precursors and possibly as energy sources. The saturated acids

Table XX. Mean individual fatty acid percentages of total FAME on day 10 in poisoned populations and their depleted media.

Fatty Acid	40 mg KPCP/l Populations N = 6	40 mg KPCP/l Day 10 Media N = 6	80 mg KPCP/l Populations N = 7	80 mg KPCP/l Day 10 Media N = 7
8:0	0.00	0.00	0.00	0.00
10:0	0.00	0.00	0.00	0.01
12:0	0.03	0.03	0.05	0.03
14:0	0.90	0.92	0.95	0.86
15:0	0.21	0.31	0.15	0.22
16:0	15.46	17.90	15.58	17.37
17:0	0.00	0.48	0.59	0.54
18:0	6.85	13.92	7.46	13.73
20:0	0.32	0.05	0.48	0.04
22:0	0.04	0.41	0.03	0.12
br13:0	0.04	0.05	0.01	0.03
br14:0	0.18	0.16	0.40	0.53
br15:0	0.14	0.14	0.10	0.14
br16:0	0.04	0.08	0.02	0.13
br17:0	0.91	0.38	0.41	0.25
14:1	0.26	0.12	0.27	0.14
15:1	0.53	0.36	0.30	0.05
16:1ω7	7.74	3.75	6.64	3.62
17:1	0.89	0.78	0.61	0.65
18:1ω9	42.11	31.81	45.70	31.40
19:1	0.02	0.00	0.00	0.00
20:1ω9	0.10	0.14	0.20	0.85
16:2ω7	0.42	0.00	0.00	0.00
17:2	0.00	0.00	0.00	0.00
18:2ω9	9.50	18.46	7.14	19.10
20:2ω9	0.73	0.30	0.31	0.19
18:3ω3	1.74	0.59	1.50	0.45
20:3ω9	0.42	0.49	0.37	0.27
20:3ω6	1.54	1.40	1.43	1.64

Continued

Table XX.

Fatty Acid	40 mg KPCP/1 Populations N = 6	40 mg KPCP/1 Day 10 Media N = 6	80 mg KPCP/1 Populations N = 7	80 mg KPCP/1 Day 10 Media N = 7
18:4ω3	0.46	1.41	0.51	1.14
20:4ω6	3.76	3.33	3.94	3.44
22:4ω6	0.29	0.11	0.54	0.41
20:5ω3	0.87	0.62	1.03	0.97
22:5ω3	1.02	0.48	1.51	0.73
22:6ω3	0.62	0.33	0.92	0.63
A	1.41	0.14	0.63	0.08
B	0.24	0.16	0.00	0.00
C	0.02	0.04	0.12	0.16
D	0.15	0.24	0.04	0.00
E	0.00	0.00	0.04	0.05
Fatty Acid Mass	6,257 μg	9,538 μg	5,100 μg	12,031 μg

16:0 and 18:0 which predominate in the media were catabolized to the greatest extent.

Populations exposed to 80 mg KPCP/1 reduced the percent contribution of saturated acids in the medium from 42.5 percent to 32.9 percent while maintaining 25.3 percent in the populations. The handling of saturated fatty acids paralleled that of populations exposed to 40 mg KPCP/1, the major difference being a slightly greater retention of acid 18:0 in the cells of populations exposed to 80 mg KPCP/1.

Branch Chain Fatty Acids

Populations exposed to 40 mg KPCP/1 increased the percentage composition of branched chain acids in the medium from 0.5 percent to 0.8 percent while retaining 1.3 percent in the cells over the ten day growth period. All of the branched acids increased in percentage contribution to the media FAME with the exception of br15:0 and br16:0. These same two acids did not increase in relative percentage in the cell populations over that of day zero medium and the proportion of acid br16:0 definitely decreased in the populations. Acid br17:0 accounted for most of the increase in populations and media.

Populations poisoned with 80 mg KPCP/1 increased the percentage contribution of branched chain acids in the media from

0.5 percent to 1.1 percent while retaining 0.9 percent in the cells. These data indicate that reduced catabolism took place, but not as much reduced as in populations poisoned with 40 mg KPCP/1. Acids br14:0 and br17:0 accounted for nearly all of the percentage change. Acids br16:0 was again reduced to a low percentage in cells, but the media were not selectively depleted.

Monene Fatty Acids

Populations poisoned with 40 mg KPCP/1 decreased the percentage of monene acids in the medium from 38.5 percent to 37.0 percent and increased the percentage in the populations to 51.6 percent of the total FAME. The monene acids of 17 carbon chain and less increased their percentage contributions in the medium over the ten day growth period. Those monene acids of 18 carbon length and greater were selectively depleted from the medium during the growth period. The increase in proportions of monene fatty acids was marked in the cell population fats. All of the monene acids had a higher percentage contribution to FAME in the populations than they had in day zero medium with the exception of 20:1. These data indicate that the shorter chain monene acids are either actively synthesized by the cell populations with some loss to the medium or they are catabolized on a net basis less than the average acid and are poorly absorbed from the media. Acid 18:1 is absorbed well from the

media and preferentially stored in the cells.

Populations poisoned with 80 mg KPCP/1 decreased the percentage contributions of monene acids in the medium from 38.5 percent to 36.7 percent and increased the percentage in populations to 53.7 percent. In general, the percentage composition of the monene acids of populations and media paralleled those of the populations exposed to 40 mg KPCP/1. The exceptions to this for populations poisoned with 80 mg KPCP/1 are depletion in the percentage of 15:1 from the media and augmentation of the percentage of 20:1 in the media.

Diene Fatty Acids

Populations exposed to 40 mg KPCP/1 increased the percentage contributions of diene acids in the medium from 11.4 percent to 18.8 percent while maintaining a somewhat reduced percentage composition in populations of 10.6 percent. These data suggest net synthesis of diene acids over the ten day period and net excretion of 18:2 back to the medium against a concentration gradient.

Populations exposed to 80 mg KPCP/1 increased the percentage contributions of diene acids in the medium from 11.4 percent to 19.3 percent while maintaining a reduced percentage (7.4 percent) in the populations. These data probably represent a lower rate of synthesis in the cells poisoned with the higher level of KPCP. Export of

acids 18:2 and 20:2 from the populations to the media was not affected by poisoning, but the percentage retention of this acid by populations was progressively reduced by increased poison levels.

Triene Fatty Acids

Populations exposed to 40 mg KPCP/l increased the percentage contributions of the triene acids in the medium from 1.5 percent to 2.5 percent and in the populations to 3.7 percent. These data suggest net synthesis of all triene acids with some export of these acids against a concentration gradient.

Populations exposed to 80 mg KPCP/l increased the percentage contributions of triene acids from 1.5 percent to 2.4 percent in the medium and to 3.3 percent in the poisoned populations paralleling the percentages of trienes in medium and cells exposed to 40 mg KPCP/l.

Tetraene Fatty Acids

Populations exposed to 40 mg KPCP/l increased the percent contributions of tetraene acids from 3.8 percent to 4.8 percent in the medium and 4.5 percent in the populations with the increased percentage in cells and medium due primarily to the probable synthesis and export of 20:4. Acid 18:4 was probably synthesized and exported, but the percentage retained by the cell populations was considerably

below that of the medium. Acid 22:4 was selectively depleted from the medium.

Populations exposed to 80 mg KPCP/1 increased the percentage contributions of tetraene acids from 3.8 percent to 5 percent in the medium and to 5 percent in the populations. The patterns of handling these acids were similar to the patterns of populations exposed to 40 mg KPCP/1 with the exception of acid 22:4 which was reduced in net catabolism to the extent that it increased in percentage contribution in both medium and populations.

Pentene and Hexene Fatty Acids

The two pentene acids (20:5 and 22:5) comprised 1.3 percent of the total day zero medium FAME, 1.9 percent of the FAME from populations exposed to 40 mg KPCP/1 and 1.1 percent in the media depleted by these populations. Corresponding data for acid 22:6 are 0.5 percent, 0.6 percent and 0.3 percent. These data indicate the pentene and hexene acids were preferentially absorbed from the medium and stored against a gradient by the populations.

Populations exposed to 80 mg KPCP/1 increased the percentage compositions of pentene acids from 1.3 percent to 1.77 percent in the medium and to 2.5 percent in the populations. Acid 22:6 was increased in percentage composition of the medium from 0.5 percent to 0.6 percent and in populations to 0.9 percent. These data indicate

that populations exposed to 80 mg KPCP/1 preferentially concentrated and retained pentene and hexene acids from the medium against a gradient.

Unknown Fatty Acids

Acid A (possibly a monyne acid) increased its percentage contribution in the medium from 0.0 percent to 0.1 percent and constituted 1.4 percent of the total FAME in populations exposed to 40 mg KPCP/1 and 0.6 percent in populations exposed to 80 mg KPCP/1. These data indicate that acid A must have been synthesized by the populations with subsequent leakage to the medium. Populations exposed to 80 mg KPCP/1 did not increase the percentage contribution of acid A in the cells as much as did the populations exposed to 40 mg KPCP/1.

Poisoned Versus Control Populations

Table XXI contains the differences in percent composition of FAME between populations exposed to 40 mg KPCP/1 and their paired controls, between the depleted media of these two populations, between populations exposed to 80 mg KPCP/1 and their paired controls and between the depleted media of these latter two populations. The differences (poisoned minus controls) express changes in individual fatty acid percent contributions due to pentachlorophenol

Table XXI. Mean differences (poisoned minus control) of individual fatty acid percent contributions to total FAME between poisoned populations and their paired controls. Data are given for cells and media.

Fatty Acid	40 mg KPCP/1 Populations N = 6	Day 10 40 mg KPCP/1 Media N = 6	80 mg KPCP/1 Populations N = 7	Day 10 80 mg KPCP/1 Media N = 7
8:0	0.00	0.00	0.00	0.00
10:0	0.00	0.00	0.00	0.00
12:0	0.00	0.00	0.04***	-0.01*
14:0	0.02	-0.09	0.10	-0.19***
15:0	-0.01	-0.01	0.03*	-0.03*
16:0	-0.27	-0.33	0.01	0.41
17:0	0.00	0.00	-0.04	-0.18
18:0	-0.01	1.60*	0.29	1.59**
20:0	0.05**	-0.01	-0.16	-0.04
22:0	0.00	0.00	-0.04*	-0.06
br13:0	-0.03***	-0.04***	-0.04***	-0.06***
br14:0	-0.05***	-0.13***	0.05	-0.26***
br15:0	-0.01	-0.07***	0.00	-0.08***
br16:0	0.00	-0.01	-0.02	-0.01
br17:0	-0.01	-0.04***	-0.22	0.10
14:1	0.10***	0.01*	0.13***	0.03
15:1	0.08***	0.05*	0.03	-0.02*
16:1ω7	0.20	-0.29	-0.15	-0.37*
17:1	0.01	-0.10	0.01	-0.03
18:1	-0.68	-0.82	4.42***	1.10*
19:1	0.01	0.00	0.00	0.00
20:1ω9	-0.02	-0.01	-0.08	0.11
16:2ω7	0.00	0.00	0.00	0.00
17:2	0.00	0.00	0.00	0.00
18:2ω9	-0.24	-0.07	-2.92***	-1.65*
20:2ω9	-0.10	-0.10	-0.61***	0.02
18:3ω3	0.29*	-0.03	-0.17	-0.17
20:3ω9	0.00	0.05	-0.20***	0.05
20:3ω6	-0.17**	0.15***	-0.48***	0.14

Continued

Table XXI.

Fatty Acid	40 mg KPCP/1 Populations N = 6	Day 10 40 mg KPCP/1 Media N = 6	80 mg KPCP/1 Populations N = 7	Day 10 80 mg KPCP/1 Media N = 7
18:4ω3	0.08	-0.12	0.13***	-0.12*
20:4ω6	0.37*	0.50*	0.10	-0.17
22:4ω6	0.00	0.02	0.03	0.14
20:5ω3	0.06	-0.04	0.01	0.15
22:5ω3	0.09*	0.08	0.27***	-0.11
22:6ω3	0.08	0.06	0.16	0.22
A	-0.01	0.02*	-0.48**	0.02
B	0.08	0.05	0.00	0.00
C	0.01	0.04	-0.14*	-0.16
D	0.04	0.01	0.04	0.00
E	0.00	0.00	-0.10	-0.28

*Significant at $p < .05$
**Significant at $p < .01$
***Significant at $p < .005$

poisoning alone.

Saturated Fatty Acids

Poisoning cell populations with 40 mg KPCP/1 reduced the population's percentage of saturated fatty acids by 0.22 percentage units while increasing the percentage composition of the saturated fatty acids in the medium by 1.16 percentage units. Acid 20:0 was the only saturated acid with a statistically significant change in the percentage composition of 40 mg KPCP/1 poisoned cell populations. Acid 18:0 remained fairly stable in its percent contribution to cell FAME, but had the greatest change in percent contribution to the FAME of the depleted media and was the only saturated acid with a statistically significant change in the percentage composition of depleted media due to poisoning.

Populations poisoned with 80 mg KPCP/1 increased the percentage of saturated acids over those of control populations by 0.23 percentage units with acids 12:0 and 15:0 increasing with statistical significance. The media of populations poisoned with 80 mg KPCP/1 increased their percentage of saturated acids over the media of control populations by 1.49 percentage units with acids 12:0, 14:0 and 15:0 decreasing their percentages and acid 18:0

increasing its percentage (statistically significant). The change of greatest magnitude in both poisoned groups compared to their control groups was the increased percentage of acid 18:0 in the depleted media from poisoned populations.

Branch Chain Fatty Acids

Populations exposed to 40 mg KPCP/1 and their depleted media had lower percentages of the branch chain fatty acids (0.10 and 0.29 percentage units respectively) compared to control values. Reduced values of br13:0 and br14:0 were statistically significant in the populations and reduced values of br13:0, br14:0, br15:0 and br17:0 were statistically significant in the media of poisoned populations.

Populations exposed to 80 mg KPCP/1 and their depleted media also had lower percentages of branch chain fatty acids by 0.24 and 0.31 percentage units respectively. The value of acid br13:0 was lower significantly in the poisoned populations and the values of acids br13:0, br14:0 and br15:0 were lower significantly in depleted media from the poisoned populations.

Generally, poisoning the cell populations caused a reduction in the percentages of branch chain fatty acids in both the populations

and their depleted media. These data would support the interpretation of increased catabolic utilization of branch chain acids by poisoned cell populations.

Monene Fatty Acids

Although acid 18:1 comprised more than 40 percent of the total cellular fatty acid and more than 30 percent of the total fatty acid found in the medium, poisoning cell populations at 40 mg KPCP/l did not alter the percent contribution of 18:1 substantially in either the cell population or depleted medium. Poisoned populations contained less monene acids by 0.30 percentage units than did controls and the depleted media from poisoned populations contained 1.16 percentage units less monene acids. Acids 14:1 and 15:1 had higher percentage contributions of statistical significance in poisoned populations and in depleted media from these populations than did control populations and media.

Populations poisoned with 80 mg KPCP/l contained 4.36 percentage units more monene acids than did controls for which the major contributing acid was 18:1. Acids 14:1 and 18:1 were higher in their percentage contributions to the FAME of poisoned populations. Depleted media from populations poisoned with 80 mg KPCP/l contained 0.82 percentage units more monene acids than

did the paired controls. A higher percentage content of acid 18:1 and lower percentage contents of acids 15:1 and 16:1 were of statistical significance. These data represent a marked, selective change in the metabolism of acid 18:1 by populations exposed to 80 mg KPCP/1 from that of populations exposed to 40 mg KPCP/1 or control populations.

Diene Fatty Acids

The diene acids from populations exposed to 40 mg KPCP/1 and from the depleted media of these populations maintained percentage compositions similar to paired control populations and media. There was a drop of 0.34 percentage units for diene acids in the populations and a drop of 0.17 percentage units of diene acids in depleted media from these populations due to 40 mg KPCP/1 poisoning.

At the higher poisoning level of 80 mg KPCP/1, however, a drop of total diene acids of 3.53 percentage units was experienced by poisoned populations with a drop of 1.65 percentage units of diene acids composing the FAME of depleted media. These reduced diene percentages in 80 mg KPCP/1 poisoned populations were due to the highly statistically significant differences in acids 18:2 and 20:2 in

poisoned cell populations and acid 18:2 in media depleted by the populations poisoned at the higher KPCP level.

Triene Fatty Acids

Populations poisoned with 40 mg KPCP/1 experienced a total gain of 0.12 percentage units over paired controls which was due to a statistically significant gain in the percent contribution of acid 20:3 ω6 to the population's FAME. Depleted media from these poisoned populations increased the percentage contributions of triene acids by 0.17 percentage units, an event due almost entirely to a statistically significant gain of acid 20:3 ω6.

Populations poisoned with 80 mg KPCP/1 had a decreased percentage of triene acids by 0.85 percentage units over paired controls with little change in the triene percent FAME contribution in depleted media from these populations. Acids 20:3 ω9 and 20:3 ω6 contributed smaller percentages of total FAME in cell populations poisoned with 80 mg KPCP/1 than in paired controls.

Tetraene Fatty Acids

Populations exposed to 40 mg KPCP/1 and depleted media from these populations had slightly higher percentages of tetraene acids by 0.45 and 0.40 percentage units respectively. Both of these changes occurred predominantly because of statistically

significant increases in the percentage of acid 20:4.

Populations exposed to 80 mg KPCP/1 had a slightly higher percent contribution of tetraene acids in the cells but slightly lower contributions by tetraene acids in the media depleted by these populations. The differences of acid 18:4 in poisoned populations and their depleted media compared to paired controls were statistically significant.

Pentene Fatty Acids

Populations exposed to 40 mg KPCP/1 and depleted media from these populations had slightly higher percentage contributions of pentene acids by 0.15 and 0.12 percentage units respectively than did paired control populations plus depleted media. Only the increased contribution of acid 22:5 in cell population FAME was of statistical significance.

Populations exposed to 80 mg KPCP/1 and depleted media from these populations also had slightly higher percentage contributions of pentene acids by 0.28 and 0.04 percentage units respectively. Acid 22:5 had a statistically significant gain in its percent contribution to population FAME which was greater than the gain in populations exposed to 40 mg KPCP/1.

Unknown Fatty Acids

Statistically significant differences between FAME from poisoned and control populations occurred for fatty acid A (a possible monyne) and fatty acid C. Fatty acid A had a higher percentage in the depleted media from populations poisoned with 40 mg KPCP/1 than in paired control populations. Both acids A and C were lower in percentage in populations poisoned with 80 mg KPCP/1 than in the populations of paired controls. These reductions were most likely due to a decreased synthesis of these acids.

Estimation of Individual Fatty Acid Masses

Estimations of the mass of each individual fatty acid were determined by multiplying the total FAME mass times the percentage contributions of individual acids as determined by gas chromatography. Table XXII contains the mean masses of individual fatty acids determined from seven samples of day zero medium, 19 samples of control populations after ten days of growth and 13 samples of medium depleted by the ten day growth of control populations. The total mass of each acid which was present and available to cells on day zero was determined by adding the mass of each acid present in day zero medium to the mass present in 144 million cells. The mass of each acid in 144 million cells was estimated by the product of

Table XXII. Mean masses of individual fatty acids in day 0 medium, day 10 control populations and day 10 depleted media. The second column gives the available mass of each fatty acid on day 0 obtained by summation of the masses in medium and in cells.

Fatty Acid	Day 0 Medium N = 7	Day 0 Mass Available N = 7	Day 10 Control Populations N = 19	Day 10 Control Media N = 13
8:0	0 μg	0 μg	0 μg	0 μg
10:0	2	2	2	1
12:0	22	22	2	4
14:0	190	219	68	104
15:0	36	42	15	29
16:0	4268	4713	1043	1766
17:0	84	90	16	61
18:0	2395	2611	511	1221
20:0	1	15	33	7
22:0	16	18	4	28
Subtotal	7014	7732	1694	3221
br13:0	0 μg	2 μg	6 μg	9 μg
br14:0	6	17	25	54
br15:0	29	34	12	22
br16:0	3	5	5	12
br17:0	35	61	62	28
Subtotal	73	119	110	125
14:1	8 μg	12 μg	10 μg	11 μg
15:1	16	30	32	18
16:1ω7	458	665	487	407
17:1	71	97	62	79
18:1ω9	5774	6918	2680	3160
19:1	0	2	5	0
20:1ω9	32	38	14	45
Subtotal	6359	7762	3290	3720

Continued

Table XXII.

Fatty Acid	Day 0 Medium N = 7	Day 0 Mass Available N = 7	Day 10 Control Populations N = 19	Day 10 Control Media N = 13
16:2ω7	0 μg	6 μg	15 μg	0 μg
17:2	0	4	9	0
18:2ω9	1892	2187	695	1986
20:2ω9	2	32	71	27
Subtotal	1894	2229	790	2013
18:3ω3	25 μg	74 μg	115 μg	63 μg
20:3ω9	15	35	48	34
20:3ω6	202	264	148	138
Subtotal	242	373	311	235
18:4ω3	132 μg	146 μg	32 μg	141 μg
20:4ω6	455	569	270	321
22:4ω6	42	56	34	18
Subtotal	629	771	336	480
20:5ω3	90 μg	123 μg	78 μg	73 μg
22:5ω3	121	160	93	60
Subtotal	211	283	171	133
22:6ω3	75 μg	100 μg	58 μg	35 μg
A	7 μg	52 μg	108 μg	10 μg
B	0	1	32	5
C	12	14	7	18
D	0	1	2	11
E	5	6	0	19
Subtotal	24	74	149	63
Total	16,520 μg	19,445 μg	6,884 μg	10,051 μg

the mass of each acid present in the day ten control populations and the ratio of the number of cells involved (144/339).

Control Populations

The values of fatty acid masses as presented in Table XXII are largely self explanatory. Some acids such as 16:0 were depleted from the medium by the cell population over the ten day period by catabolic destruction and by cellular incorporation. Other acids such as 18:2 and 18:3 accumulated in the medium over a ten day period as well as in the cell population indicating a net synthesis of these acids and subsequent loss from the cells to the medium.

Populations Exposed to 40 mg KPCP/1

Fatty acid masses in day ten populations exposed to 40 mg KPCP/1 and in their depleted media occur in Table XXIII as data basic to subsequent comparisons.

Figures 3 and 4 graphically compare the fatty acid masses in populations exposed to 40 mg KPCP/1 (ordinate) and in their paired control populations (abscissa). Figure 4 compares acids of greater mass and Figure 3 compares acids of lesser mass. Line 1 lies at a 45 degree angle through the origin. A point on this line would represent an acid occurring in equal quantities in poisoned and control populations. Line II is between the origin and point 6608, 6257 (total

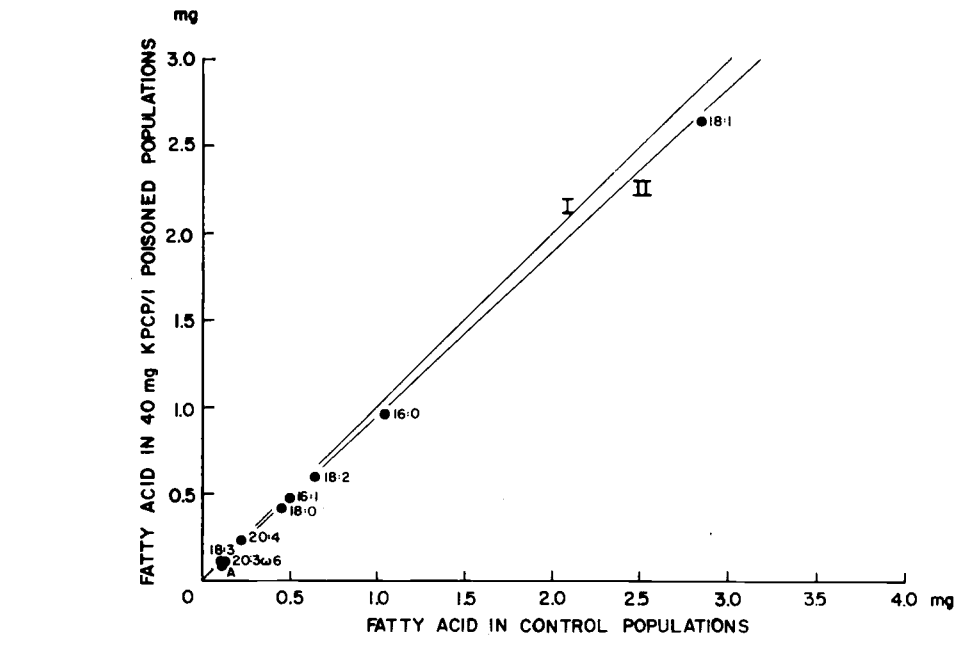
Table XXIII. Mean masses of individual fatty acids on day 10 in poisoned populations and their depleted media.

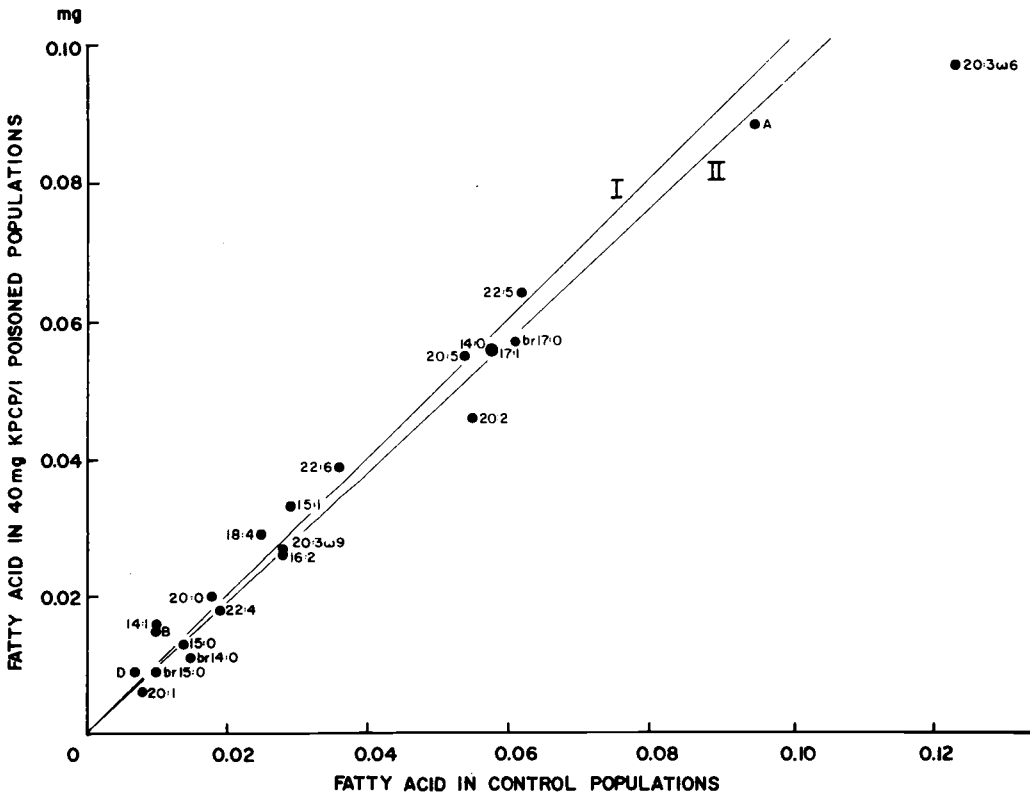
Fatty Acid	40 mg KPCP/1 Populations N = 6	40 mg KPCP/1 Day 10 Media N = 6	80 mg KPCP/1 Populations N = 7	80 mg KPCP/1 Day 10 Media N = 7
8:0	0 µg	0 µg	0 µg	0 µg
10:0	0	1	0	1
12:0	2	3	3	4
14:0	56	88	48	104
15:0	13	30	8	27
16:0	966	1696	792	2101
17:0	0	46	30	65
18:0	428	1318	382	1658
20:0	20	4	25	5
22:0	2	37	1	13
Subtotal	1,487	3,223	1,289	3,978
br13:0	3 µg	5 µg	1 µg	4 µg
br14:0	11	15	21	64
br15:0	9	14	5	17
br16:0	2	8	1	17
br17:0	57	37	21	33
Subtotal	82	79	49	135
14:1	16 µg	12 µg	14 µg	17 µg
15:1	33	34	16	5
16:1ω7	484	362	339	436
17:1	56	73	31	79
18:1ω9	2635	3025	2319	3776
19:1	1	0	0	0
20:1ω9	6	13	10	106
Subtotal	3,231	3,519	2,729	4,419

Continued

Table XXIII.

Fatty Acid	40 mg KPCP/l Populations N = 6	40 mg KPCP/l Day 10 Media N = 6	80 mg KPCP/l Populations N = 7	80 mg KPCP/l Day 10 Media N = 7
16:2ω7	26 µg	0 µg	0 µg	0 µg
17:2	0	0	0	0
18:2ω9	596	1796	365	2281
20:2ω9	46	27	16	21
Subtotal	668	1823	381	2302
18:3ω3	109 µg	55 µg	77 µg	55 µg
20:3ω9	27	44	19	32
20:3ω6	96	133	74	197
Subtotal	232	232	170	284
18:4ω3	29 µg	134 µg	26 µg	137 µg
20:4ω6	237	320	204	411
22:4ω6	18	10	28	51
Subtotal	284	464	258	599
20:5ω3	55 µg	60 µg	53 µg	115 µg
22:5ω3	64	48	78	88
Subtotal	119	108	131	203
22:6ω3	39 µg	33 µg	48 µg	74 µg
A	88 µg	13 µg	33 µg	9 µg
B	15	14	0	0
C	1	3	6	19
D	9	21	2	0
E	0	0	0	8
Subtotal	113	51	41	36
Total	6,257 µg	9,538 µg	5,100 µg	12,031 µg





Figures 3 & 4. Mean fatty acid masses in 40 mg KPCP/1 poisoned populations vs. paired control populations. A point on line I would represent an acid with equal masses in control and poisoned populations. Fatty acids were reduced by KPCP. A point on line II would represent an acid whose reduction was proportional to the total reduction of FAME in the poisoned populations. Figure 4 is an extension of Figure 3. Note change in dimensions.

fatty acid masses in control and poisoned populations). The smaller mass of fatty acids was accumulated by poisoned cells. A point lying on this line would represent an acid reduced proportionally to the average fatty acid reduction due to poisoning.

The points for several acids (18:1, 16:0, 18:2, 20:3 ω6, 18:3, 18:0 and br14:0) lie below line I with statistical significant (Table XXIV, Figures 3 and 4) indicating that at least these acids have been reduced in absolute amounts under 40 mg KPCP/l poisoning. All of the points in Figure 4 lie close to line II. Most of the major fatty acids of populations exposed to 40 mg KPCP/l have less mass than control populations by approximately the same ratio as the average mass of fatty acids in the two populations.

The points for acids 20:3 ω6 and br14:0 lie below line I of Figure 3 with statistical significance (Table XXIV) indicating that these acids occur in significantly less quantity in populations due to 40 mg KPCP/l poisoning. Both of these acids are also below line II indicating that poisoning reduces their quantities by more than the average proportion. Acids 15:1, 20:0 and 14:1 lie above line I and therefore above line II with statistical significance indicating that poisoning populations with 40 mg KPCP/l causes an increased retention of these acids in the populations.

Table XXIV contains the mean differences (poisoned minus control) of fatty acid masses between populations poisoned with 40

Table XXIV. Mean differences (poisoned minus control) and standard deviations of fatty acid masses between poisoned populations and media and their paired controls.

Fatty Acid	40 mg KPCP/1 Populations N = 6	40 mg KPCP/1 Day 10 Media N = 6	80 Mg KPCP/1 Populations M = 7	80 mg KPCP/1 Day 10 Media N = 7
8:0	0 ± 0 µg	0 ± 0 µg	0 ± 0 µg	0 ± 0 µg
10:0	0 ± 0	0 ± 1	0 ± 0	0 ± 1
12:0	0 ± 1	0 ± 3	2 ± 0***	0 ± 1
14:0	-2 ± 5	-13 ± 24	-9 ± 12*	-3 ± 14
15:0	-1 ± 1*	-2 ± 8	0 ± 2	1 ± 2
16:0	-72 ± 88*	-125 ± 239	-243 ± 165***	389 ± 259***
17:0	0 ± 0	-2 ± 6	-12 ± 21	-9 ± 44
18:0	-23 ± 6***	87 ± 230	-100 ± 64***	447 ± 315***
20:0	2 ± 1**	-1 ± 2	-19 ± 29	-2 ± 16
22:0	0 ± 4	-3 ± 28	-3 ± 2**	-3 ± 13
Subtotal	-96	-59	-386	829
br13:0	-2 ± 0***	-4 ± 2***	-3 ± 1***	-6 ± 2***
br14:0	-4 ± 2***	-14 ± 6***	-3 ± 4*	-15 ± 4***
br15:0	-1 ± 1	-8 ± 6*	-2 ± 3	-6 ± 6*
br16:0	0 ± 1	-1 ± 3	-2 ± 3	3 ± 12
br17:0	-4 ± 6	-6 ± 6*	-22 ± 29*	19 ± 57
Subtotal	-11	-33	-32	-5
14:1	6 ± 2***	1 ± 3	5 ± 2***	6 ± 7*
15:1	3 ± 1***	4 ± 5	-3 ± 7	-1 ± 2
16:1ω7	-13 ± 31	-42 ± 94	-114 ± 65***	26 ± 45
17:1	-3 ± 5	-15 ± 15*	-9 ± 7**	9 ± 12*
18:1ω9	-195 ± 181*	-231 ± 349	-425 ± 275***	712 ± 396***
19:1	0 ± 1	0 ± 0	0 ± 0	0 ± 0
20:1ω9	-1 ± 5	-2 ± 3	-10 ± 21	30 ± 120
Subtotal	-203	-285	-556	782

Continued

Table XXIV.

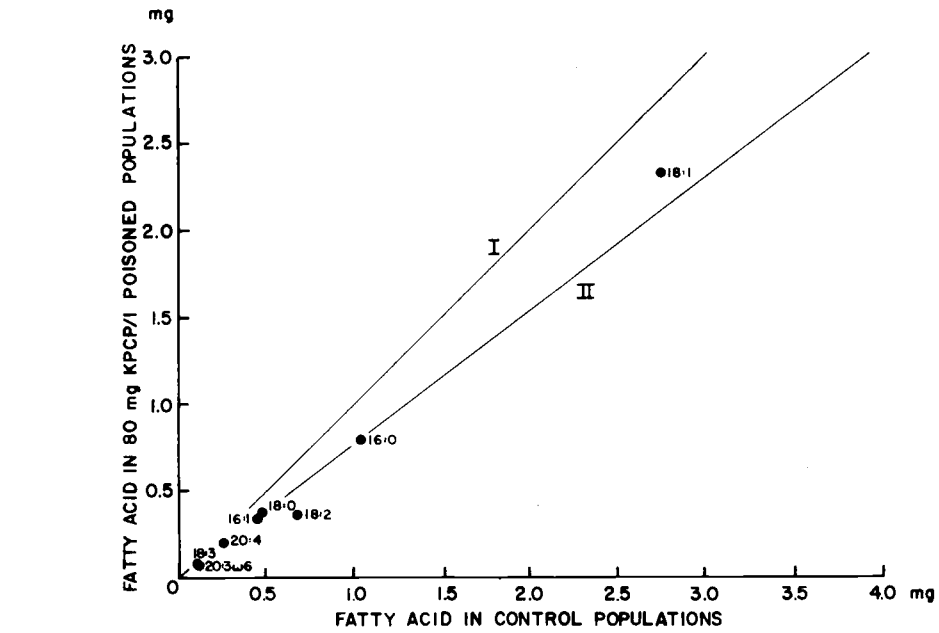
Fatty Acid	40 mg KPCP/l Populations N = 6	40 mg KPCP/l Day 10 Media N = 6	80 mg KPCP/l Populations N = 7	80 mg KPCP/l Day 10 Media N = 7
16:2ω7	-2 ± 4 μg	0 ± 0 μg	0 ± 0 μg	0 ± 0 μg
17:2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
18:2ω9	-48 ± 34**	-56 ± 551	-310 ± 148***	160 ± 219
20:2ω9	-9 ± 13	-12 ± 57	-46 ± 20***	5 ± 23
Subtotal	-59	-68	-356	165
18:3ω3	13 ± 11*	-8 ± 37	-36 ± 38*	-9 ± 37
20:3ω9	-1 ± 8	-1 ± 8	-19 ± 8***	9 ± 16
20:3ω6	-17 ± 8***	9 ± 18	-55 ± 18***	46 ± 59*
Subtotal	-5	0	-110	46
18:4ω3	4 ± 16	-19 ± 43	1 ± 5	9 ± 11*
20:4ω6	12 ± 26	37 ± 56	-54 ± 36***	50 ± 79
22:4ω6	-1 ± 3	2 ± 9	-5 ± 23	26 ± 49
Subtotal	15	20	-58	85
20:5ω3	1 ± 4	-5 ± 16	-15 ± 9***	34 ± 52
22:5ω3	2 ± 7	10 ± 23	-6 ± 15	6 ± 42
Subtotal	3	5	-21	41
22:6ω3	3 ± 6	7 ± 12	-3 ± 21	31 ± 58
A	-6 ± 4	2 ± 1***	-43 ± 29***	2 ± 12
B	5 ± 11	2 ± 42	0 ± 0	0 ± 0
C	1 ± 2	3 ± 8	-12 ± 12*	-15 ± 58
D	2 ± 10	-2 ± 28	2 ± 7	0 ± 0
E	0 ± 0	0 ± 0	0 ± 0	-30 ± 51
Subtotal	2	5	-53	-43
Total	-351 ± 322*	-443 ± 1462	-1876 ± 439***	1912 ± 1479**

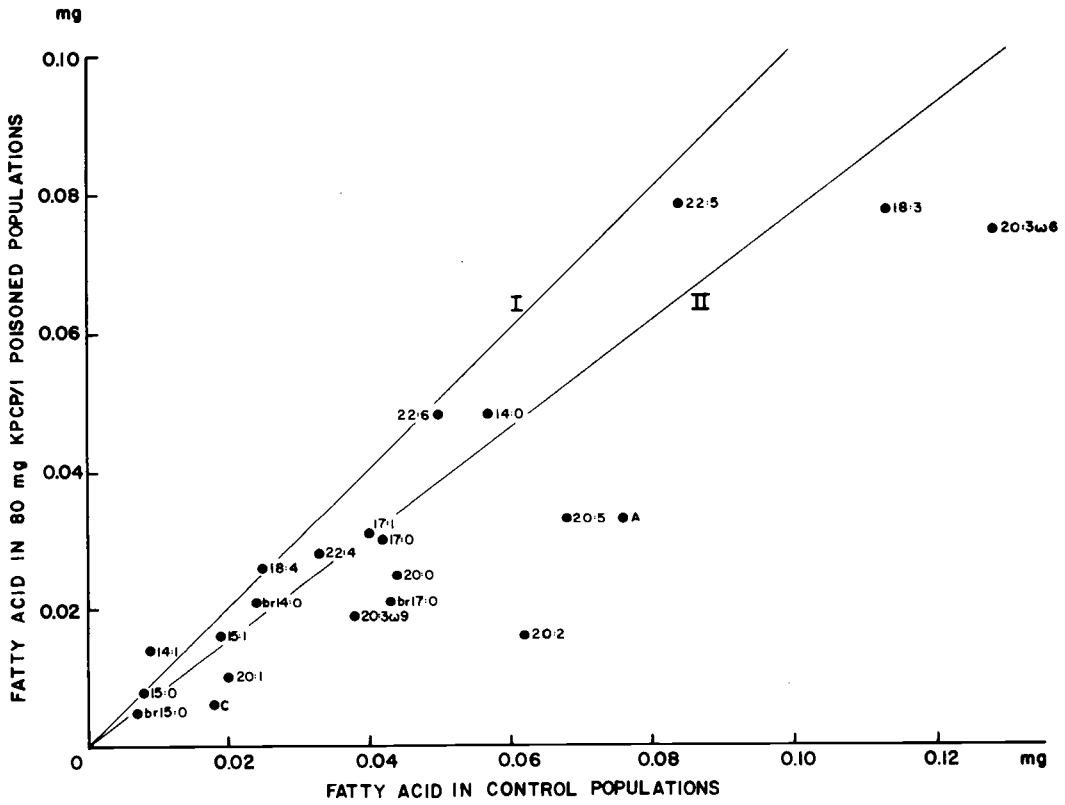
*Significant at $p<.05$
**Significant at $p<.01$
***Significant at $p<.005$

mg KPCP/1 and their paired controls and between the depleted media from these populations. Poisoning populations with 40 mg KPCP/1 caused a statistically significant decrease ($p < 0.05$) in the total fatty acid mass of populations due to poisoning and a decrease in the total fatty acid mass of depleted media after ten days of population growth.

Populations Exposed to 80 mg KPCP/1

Figures 5 and 6 graphically compare the fatty acid masses in populations exposed to 80 mg KPCP/1 (ordinate) and their paired control populations (abscissa). Figure 6 compares acids of greater mass and Figure 5 compares acids of lesser mass. Line I lies at a 45 degree angle through the origin and line II runs from the origin through point (6976, 5100) representing acids which lost an average fraction of their mass due to poisoning. In Figure 6 all of the points (acids 18:1, 16:0, 18:2, 18:0, 16:1, 20:4, 18:3, 20:3 ω6) are below line 1 with statistical significant (Table XXIV) indicating that all of the major acids were diminished in cell populations due to 80 mg KPCP/1 poisoning. In Figure 5 acids 20:3 ω6, 18:3, 14:0, 20:5, A, 20:2, 17:1, br17:0, 20:3ω9, br14:0 and C lie below line I with statistical significance indicating a diminished amount in populations exposed to 80 mg KPCP/1 compared to control populations. Only acid 14:1 lies above line I with statistical significance indicating that 14:1 is in higher quantity in poisoned populations than in control populations.





Figures 5 & 6. Mean fatty acid masses in 80 mg KPCP/1 poisoned populations vs. paired control populations. A point on line I would represent an acid with equal masses in control and poisoned populations. Fatty acids were reduced by KPCP. A point on line II would represent an acid whose reduction was proportional to the total reduction of FAME in the poisoned populations. Figure 6 is an extension of Figure 5. Note change in dimensions.

Fatty Acid Gains (Losses) from Cells Plus Media

The gains or losses in mass of total and individual fatty acid methyl esters produced by cell populations over a ten day period were calculated from the data presented. The sum of the masses of FAME in day zero medium and in the cell inoculum estimated the mass of fatty acid present on day zero. The sum of fatty acid masses determined from day ten populations and their depleted media estimated the mass of fatty acid present on day ten. Differences between fatty acid masses available on day zero and masses present on day ten determined the extent to which each acid was gained or lost over the ten day period (net synthesis or net catabolism).

Control Populations

Table XXV includes the mean masses gained or lost (micrograms) by 13 control populations, their standard deviations and the statistically significant levels of gains or losses. Control populations destroyed an average 2.744 mg of FAME during the ten day period. This loss was statistically significant ($p < 0.005$) indicating that control cell populations catabolized fatty acids.

Saturated Fatty Acids

Control populations lost an average of 2.884 mg of

Table XXV. Means and standard deviations of fatty acid masses gained (or lost) in populations plus their media over a ten day period for control populations and poisoned populations.

Fatty Acid	Control N = 13	40 mg KPCP/1 N = 6	80 mg KPCP/1 N = 7
8:0	0 ± 0 μg	0 ± 0 μg	0 ± 0 μg
10:0	-1 ± 1***	-2 ± 1***	-1 ± 1*
12:0	-17 ± 2***	-18 ± 2***	-16 ± 1***
14:0	-57 ± 26***	-75 ± 20***	-66 ± 32***
15:0	-2 ± 12	1 ± 9	-7 ± 9*
16:0	-1914 ± 274***	-2051 ± 145***	-1820 ± 462***
17:0	-6 ± 49	-45 ± 4***	5 ± 21
18:0	-923 ± 169***	-865 ± 171***	-572 ± 443**
20:0	23 ± 28**	9 ± 3***	16 ± 12**
22:0	13 ± 36	22 ± 60	-4 ± 13
Subtotal	-2884	-3024	-2465
br13:0	11 ± 4***	5 ± 2***	2 ± 2*
br14:0	59 ± 33***	9 ± 9*	68 ± 13***
br15:0	-4 ± 6*	-11 ± 36	-12 ± 5***
br16:0	10 ± 7***	5 ± 4**	13 ± 17*
br17:0	18 ± 33*	33 ± 10***	-6 ± 64
Subtotal	94	41	65
14:1	9 ± 6***	16 ± 5***	19 ± 12***
15:1	12 ± 20*	38 ± 11***	-8 ± 9*
16:1ω7	216 ± 129***	180 ± 111**	110 ± 88**
17:1	30 ± 41*	32 ± 43	13 ± 21
18:1ω9	-982 ± 509***	-1258 ± 427***	-823 ± 644**
19:1	-2 ± 1***	-1 ± 3	-2 ± 0
20:1ω9	23 ± 58	-19 ± 4***	78 ± 75*
Subtotal	-694	-1012	-613

Continued

Table XXV.

Fatty Acid	Control N = 13	40 mg KPCP/l N = 6	80 mg KPCP/l N = 7
16:2ω7	7 ± 19 μg	20 ± 21* μg	-6 ± 0 μg
17:2	-4 ± 0	-4 ± 0	-4 ± 0
18:2ω9	470 ± 517***	205 ± 675	460 ± 436*
20:2ω9	53 ± 31***	40 ± 56	4 ± 11
Subtotal	526	261	454
18:3ω3	95 ± 46***	90 ± 22***	59 ± 46
20:3ω9	32 ± 42**	36 ± 65	16 ± 13**
20:3ω6	-4 ± 46	-34 ± 32*	6 ± 89
Subtotal	123	92	81
18:4ω3	19 ± 53	17 ± 67	17 ± 27
20:4ω6	-2 ± 94	-13 ± 114	46 ± 118
22:4ω6	-12 ± 30	-28 ± 11***	23 ± 44
Subtotal	5	-24	86
20:5ω3	13 ± 26*	-8 ± 33	45 ± 64
22:5ω3	-25 ± 60	-48 ± 58*	6 ± 37
Subtotal	-12	-56	51
22:6ω3	-21 ± 36*	-28 ± 38	22 ± 54
A	42 ± 32***	50 ± 27***	-10 ± 21
B	8 ± 17	27 ± 54	-1 ± 0
C	14 ± 40	-10 ± 8*	11 ± 33
D	13 ± 23*	29 ± 40	2 ± 7
E	20 ± 46	-6 ± 0	3 ± 25
Subtotal	97	90	5
Total	-2744 ± 1528***	-3650 ± 1438***	-2313 ± 2208*

*Significant at $p < .05$
**Significant at $p < .01$
***Significant at $p < .005$

saturated fatty acids over the ten day period. This loss accounts for more than the total fatty acid loss; thus, the saturated fatty acids were by far the dominant fatty acid substrates for catabolism and energy production from fats. Losses of acids 16:0 and 18:0 accounted for most of the saturated acid loss reflecting their preponderance in avilable saturated fatty acids. Statistically significant losses occurred in acids 10:0, 12:0, 14:0, 16:0 and 18:0 with a probability of occurrence by chance less than 0.005. Acid 20:0 gained in quantity over the ten day period with a chance probability less than 0.01.

Branch Chain Fatty Acids

Branch chain fatty acids gained a total of 94 μg over the ten day period. Statistically significant gains occurred with acids br13:0, br14:0 and br16:0 ($p < 0.005$) and br17:0 ($p < 0.05$). A statistically significant loss of acid br15:0 also occurred ($p < 0.05$).

Monene Fatty Acid

Control populations lost 0.982 mg of monene acids due almost entirely to a large loss of acid 18:1. Most monene acids increased in content over the ten day period. Statistically significant gains were found for acids 14:1 and 16:1 ($p < 0.005$) and 15:1 and 17:1 ($p < 0.05$). Statistically significant losses were determined for acids 18:1 and 19:1 ($p < 0.005$).

Polyunsaturated Fatty Acids

Diene acids gained a total of 0.526 mg over the ten day period. Acid 18:2 gained more mass than any other acid. Both acids 18:2 and 20:2 gained statistically significant amounts ($p < 0.005$). Control populations produced a net gain of 0.123 mg of triene acids. Acid 18:3 accounted for most of the gain with a probability for chance occurrence less than 0.005. The gain of acid 20:3 ω9 was also statistically significant ($p < 0.01$). The higher molecular weight polyunsaturated fatty acids were generally present in small quantity and their relatively small gains or losses were not generally statistically significant. A statistically significant gain of acid 20:5 ($p < 0.05$) did occur, however.

Unknown Fatty Acids

Control populations produced statistically significant net gains of two unidentified acids, A ($p < 0.005$) and D ($p < 0.05$).

Summary

It is interesting to note that while control populations produced net losses to the point of statistical significance in eight fatty acids, none of these acids were polyunsaturated. Control populations produced statistically significant net losses in eight fatty acids

over a ten day period. These acids follow in descending order of mass lost: 16:0, 18:1, 18:0, 14:0, 12:0, br15:0, 19:1 and 10:0. Control populations produced statistically significant net gains in 16 acids over the ten day period. These acids follow in descending order of mass gained: 18:2, 16:1, 18:3, br14:0, 20:2, 20:3 ω9, 17:1, D, 20:5, 20:0, br17:0, 15:1, br13:0, br16:0, and 14:1 (Table XXV).

Populations Exposed to 40 mg KPCP/1

Populations poisoned with 40 mg KPCP/1 destroyed an average 3.650 mg of fatty acid methyl esters over a ten day period. This loss was statistically significant with a probability of chance occurrence less than 0.005.

Populations exposed to 40 mg KPCP/1 lost 0.794 mg total FAME more than did their paired control populations. The excess loss of total FAME due to poisoning was not statistically significant; however, over the ten day period, several individual fatty acids did gain or lose amounts differing significantly between poisoned and control populations. Table XXVI and Figures 7 and 8 compare the mean values of fatty acid masses gained or lost in populations plus their media over a ten day period for populations exposed to 40 mg KPCP/1 and their paired controls. Figures 7 and 8 graphically display the gains or losses of each fatty acid. Points occurring in the upper right quadrant represent fatty acids gained in both poisoned and

Table XXVI. Mean differences (poisoned minus control) and standard deviations of differences of fatty acid masses in populations plus their media on day 10 between control and poisoned populations.

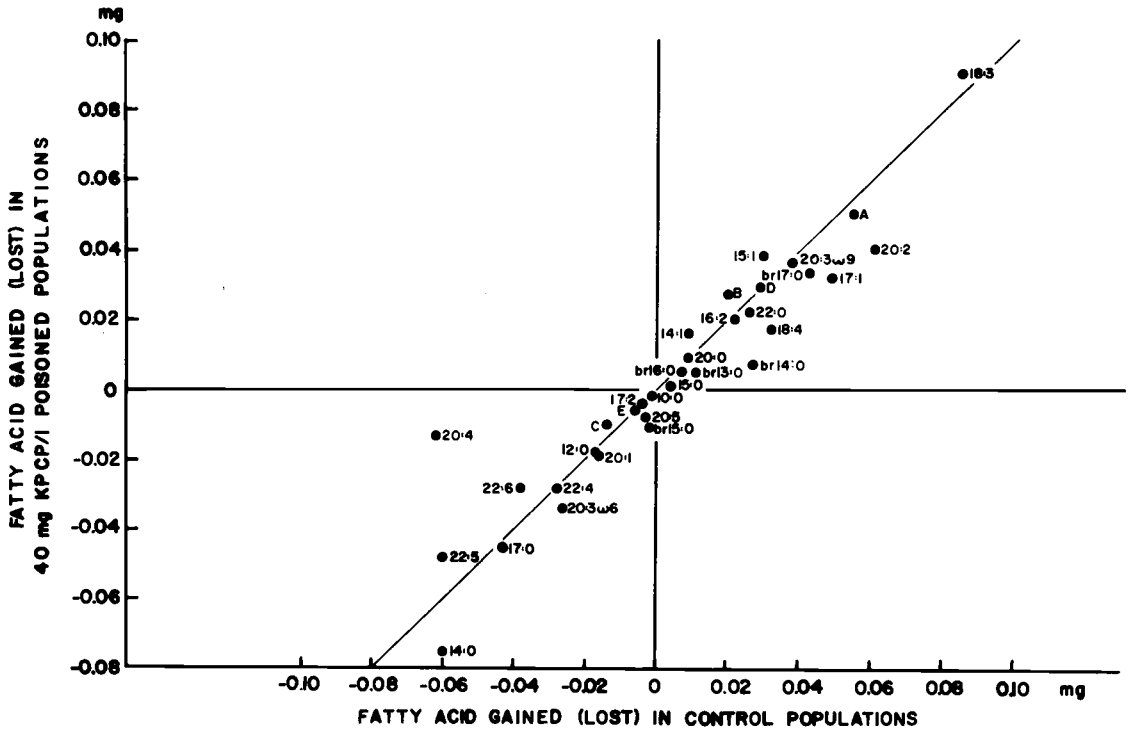
Fatty Acid	40 mg KPCP/l N = 6	80 mg KPCP/l N = 7
8:0	0 ± 0 μg	0 ± 1 μg
10:0	0 ± 1	0 ± 1
12:0	0 ± 3	2 ± 1**
14:0	-15 ± 26	-11 ± 11*
15:0	-3 ± 7	0 ± 4
16:0	-197 ± 262	146 ± 303
17:0	-2 ± 6	-21 ± 50
18:0	64 ± 289	347 ± 293*
20:0	1 ± 2	-20 ± 29
22:0	-4 ± 29	-5 ± 15
br13:0	-6 ± 2***	-9 ± 3***
br14:0	-18 ± 7***	-18 ± 6***
br15:0	-9 ± 7*	-8 ± 5***
br16:0	-2 ± 4	1 ± 10
br17:0	-10 ± 10*	-2 ± 44
14:1	7 ± 3***	11 ± 9**
15:1	7 ± 6*	-4 ± 7
16:1ω7	-55 ± 90	-89 ± 96*
17:1	-18 ± 13*	0 ± 16
18:1ω9	-426 ± 465*	287 ± 516
19:1	0 ± 1	0 ± 0
20:1ω9	-3 ± 6	21 ± 116
16:2ω7	-2 ± 4 μg	0 ± 0 μg
17:2	0 ± 0	0 ± 0
18:2ω9	-103 ± 551	-150 ± 301
20:2ω9	-21 ± 63	-42 ± 32**

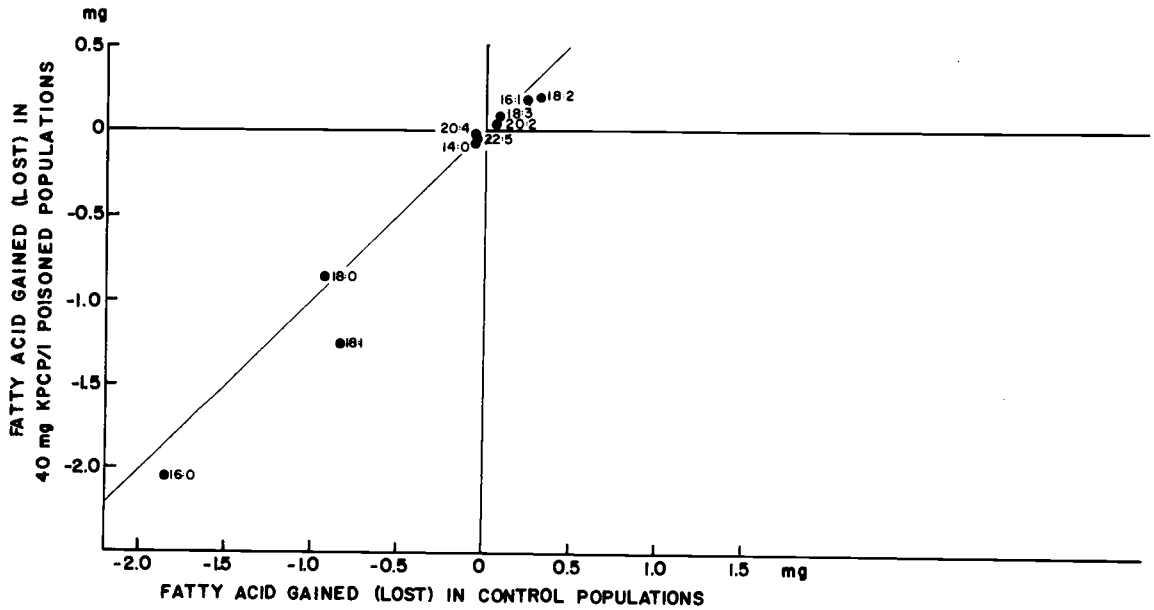
Continued

XXVI.

Fatty Acid	40 mg KPCP/l N = 6	80 mg KPCP/l N = 7
18:3ω3	5 ± 37	-44 ± 37**
20:3ω9	-2 ± 14	-10 ± 13*
20:3ω6	-8 ± 23	-9 ± 48
18:4ω3	-15 ± 53	10 ± 12*
20:4ω6	49 ± 74	-4 ± 78
22:4ω6	1 ± 9	21 ± 57
20:5ω3	-4 ± 18	18 ± 51
22:5ω3	1 ± 27	1 ± 55
22:6ω3	1 ± 15	28 ± 54
A	-5 ± 4*	-40 ± 32**
B	7 ± 48	0 ± 0
C	4 ± 8	-27 ± 53
D	0 ± 35	2 ± 7
E	0 ± 0	-39 ± 49*
Total	-794 ± 1595	334 ± 1521

*Significant at $p < .05$
**Significant at $p < .01$
***Significant at $p < .005$





Figures 7 & 8. Mean masses of fatty acid gained (lost) in cells and media over ten days by 40 mg KPCP/1 poisoned populations vs. paired control populations. A point on the diagonal line would represent an equal gain or loss by both poisoned and control populations. Points in quadrant I represent acids whose masses were increased during culture of poisoned and control populations and points in quadrant III represent acids whose masses were reduced. Figure 8 is an extension of Figure 7.

control populations. Points occurring in the lower left quadrant represent fatty acids lost in both poisoned and control populations. There are no points in the other two quadrants indicating that no fatty acid gained in control populations but lost in poisoned populations or vice versa. A diagonal line is drawn through the origin at a 45 degree angle to the abscissa. This diagonal is the locus of points representing acids with equal gains or losses in poisoned and control populations. Points above this line indicate acids that have lost less mass in poisoned populations than they did in control populations. Points below this line represent fatty acids that experienced increased destruction or reduced synthesis due to poisoning. Figure 8 presents comparisons of fatty acids lost or gained in large quantities and Figure 7 presents data on fatty acids lost or gained in smaller quantities.

One can quickly see in Figures 7 and 8 that acids 18:3, 15:1, B, and 14:1 gained more mass in poisoned populations than in control populations (their points lie above a 45 degree angle from the origin). The gains in mass of acids 18:2, 20:2, A, 17:1, br17:0, 18:4 and br14:0 were diminished over the ten day period by 40 mg KPCP/l poisoning. Smaller amounts of acids 18:0, 22:5, 20:4, 22:6 and C were lost with 40 mg KPCP/l while larger amounts of acids 18:1, 14:0, 20:3 ω6 and br15:0 were lost than were lost in control experiments.

Values in Table XXVI are mean differences in micrograms of fatty acid gained between populations poisoned with 40 mg KPCP/l and paired control populations (poisoned minus control). A negative value could indicate either decreased gain or increased loss due to poisoning.

Saturated Fatty Acids

Poisoned populations generally lost a larger mass of saturated acids than did their paired controls. The major exception to this statement was acid 18:0 where the losses were nearly equal. None of the differences, however, were statistically significant.

Branch Chain Fatty Acids

Changes in branch chain fatty acids due to 40 mg KPCP/l poisoning were decreased gains and increased losses, generally statistically significant. Acids br13:0 and br14:0 had statistically significant decreased gains ($p < 0.005$). Acid br15:0 had an increased loss ($p < 0.005$) and acid br17:0 had a decreased gain ($p < 0.05$).

Monene Fatty Acids

Poisoning populations at 40 mg KPCP/l caused statistically significant increased gains of the shorter chain monene acids 14:1 and 15:1 ($p < 0.005$ and 0.05 respectively). Longer chain

monene acids had reduced gains or increased losses. The reduced gain of acid 17:1 and the increased loss of acid 18:1 were both statistically significant ($p < 0.05$).

Polyunsaturated Fatty Acids

KPCP led to lower gains in all the diene fatty acids but not to the extent of statistical significance. Changes in the gains of triene acids were slight and without statistical significance. Acid 18:4 gained less than in control populations. Acids 20:4 and 22:4 were eliminated less extensively in poisoned populations than in control populations, but again these differences were without statistical significance. The changes due to 40 mg KPCP/l poisoning in acids 20:5, 22:5, and 22:6 were minor and without statistical significance.

Unknown Fatty Acids

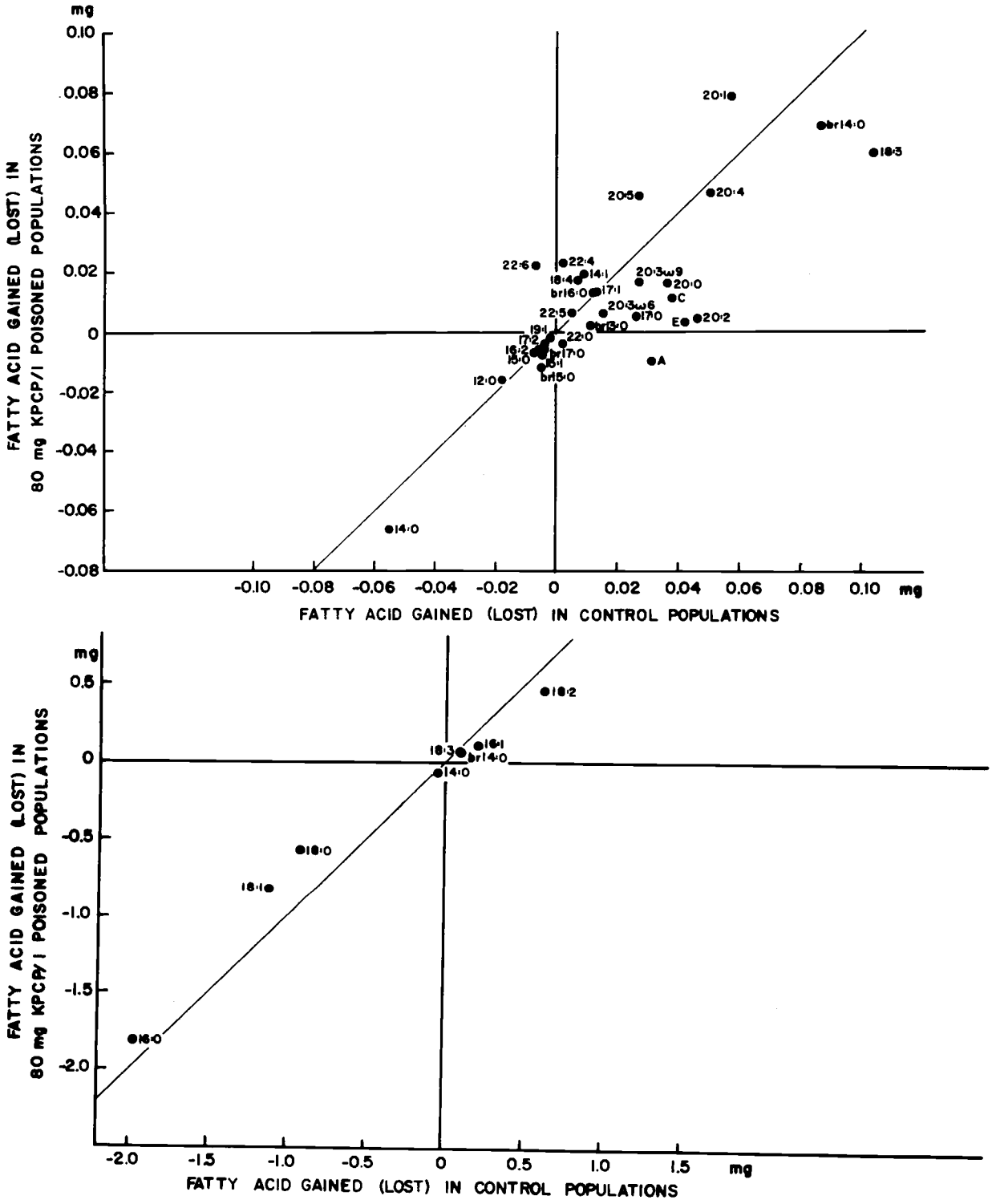
Acid A gained less in populations exposed to 40 mg KPCP/l with a probability for chance occurrence less than 0.05. Other unknown acid changes in mass were minor and not statistically significant.

Populations Exposed to 80 mg KPCP/l

Populations poisoned with 80 mg KPCP/l lost an average 2.313 mg FAME over a ten day period. This loss was statistically

significant with a probability of error less than 0.05. Although there was a net loss of fatty acid mass by poisoned populations, populations exposed to 80 mg KPCP/1 lost 0.334 mg less total FAME than did their paired control populations. The reduced loss of fatty acid was not statistically significant. It is likely that the reduced loss of fatty acids by poisoned populations was due to a reduced number of cells in these populations compared to their control populations.

Table XXVI and Figures 9 and 10 compare the mean values of fatty acid masses gained or lost in populations plus their media over a ten day period under the action of 80 mg KCPC/1 to their paired controls. Figures 9 and 10 graphically display the gains or losses of each fatty acid with Figure 9 containing comparisons of acids with greater values. The diagonal line in these figures is drawn through the origin at a 45 degree angle to the abscissa and represents acids with equal gains or losses in both control and poisoned populations. Figures 9 and 10 indicate that acids 16:0, 18:1 and 18:0 experienced a net loss in both control and poisoned populations. There was less loss of these acids in 80 mg KPCP/1 poisoned populations than in their paired controls. Acids 14:0 and br15:0 also experienced a net loss in both control and poisoned populations. The masses of acids 14:0 and br15:0 lost were greater in poisoned than in control populations (their points are below a 45 degree angle from the origin). Acids A and 22:0 experienced gains



Figures 9 & 10. Mean masses of fatty acid gained (lost) in cells and media over ten days by 80 mg KPCP/1 poisoned populations vs. paired control populations. A point on the diagonal line would represent an equal gain or loss by both poisoned and control populations. Points in quadrant I represent acids whose masses were increased during culture of poisoned and control populations and points in quadrant III represent acids whose masses were reduced. Figure 10 is an extension of Figure 9.

by control populations and losses under 80 mg KPCP/1. A large number of acids including 18:2, 16:1, br14:0, 18:3, 20:4, 20:0, 20:3 ω9, 20:2, E, C, 17:0, 20:3 ω6 and br13:0 gained mass in both poisoned and control populations, but gained less mass in the poisoned populations. There were also gains in mass of acids 20:1, 20:5, 22:4, 14:1 and 18:4 in both poisoned and control populations. These acids gained more mass in populations poisoned with 80 mg KPCP/1 than in paired control populations. Acid 22:6 lost mass in control populations but gained mass in populations poisoned with 80 mg KPCP/1.

Values expressing the mean differences (poisoned minus control) of fatty acid masses gained in populations plus their media over a ten day period between populations exposed to 80 mg KPCP/1 and their paired controls are given in Table XXVI. Many of these differences in gains due to 80 mg KPCP/1 poisoning were statistically significant. Acids 18:0 and 12:0 were lost in greater amounts by control populations than by poisoned populations and the differences were statistically significant ($p < 0.05$ and 0.01, respectively). Acids 14:0 and br15:0 were lost in greater amounts by poisoned populations than by control populations with the differences being statistically significant ($p < 0.05$ and 0.005, respectively). The statistically significant difference of acid A, ($p < 0.01$) reflects a gain in control populations and a loss in poisoned populations. The following acids gained more

mass in control than poisoned populations with the differences in gain being statistically significant: br13:0, and br14:0 ($p < 0.005$); 20:2 and 18:3 ($p < 0.01$); 16:1, 20:3 ω9 and E ($p < 0.05$). Acids 14:1 and 18:4 gained more mass in 80 mg KPCP/l poisoned populations than in control populations with the differences in gain being statistically significant ($p < 0.01$ and 0.05, respectively).

Comparative Destruction of Individual Fatty Acids

The reason for representing data for fatty acid metabolism in the complex manner of Figures 11 through 14 requires some explanation. A previous study (22) indicated that the fraction of each fatty acid that was lost or gained (mass lost or gained divided by mass available) had a relatively constant relationship to the fraction of total fatty acid mass that was lost in both control and poisoned coho salmon. Some fatty acids were lost at the same rate as the total of all fatty acids; some were lost more rapidly and some less rapidly. If the fractional net loss of the total fatty acid mass was increased due to pentachlorophenol poisoning, the fractional net loss of each individual acid increased by the same amount that the fractional loss of the total FAME increased or if a net gain had been incurred in control animals, there was a reduction in the fraction gained and the reduction was the same as the increase in the fractional loss of total FAME. These relationships appeared only when the fractional loss of fatty acids was based on an amount of fatty acid available to the fish in diet plus fish body fat on day zero. Similar relationships were found in the fatty acid metabolism of cell

populations. Again the relationships appeared only when the mass of fatty acid available was considered (when the mass of fatty acid in the day zero medium was added to the mass in the cell inoculum). The general formula for these computations of fractions plotted was:

$$[I_i + S_i - P_i - M_i] / [I_i + S_i] - \sum_{1}^{n} [I_i + S_i - P_i - M_i] / \sum_{1}^{n} [I_i + S_i]$$

= Fraction of i lost minus Fraction of total FAME lost

Where I = fatty acid mass in inoculum
S = fatty acid mass in day zero medium
P = fatty acid mass in day ten populations
M = fatty acid mass in day ten depleted medium
i = individual acids one through n.

One might question whether cellular fatty acid and medium fatty acid have the same probabilities of being transformed by cellular enzymes and can thus be lumped as "fatty acid available"; however, over the ten day period of these experiments and comparing the relatively slight differences in the net results of fatty acid metabolism in control, 40 mg KPCP/l poisoned and 80 mg KPCP/l poisoned populations this item seems to approximate the truth closely. Treating only medium fatty acid or only cellular fatty acid as the amount available does not produce a recognizable pattern. Another indication that this relationship approximates the true situation is the fact that subtraction of the fraction of total mass of fatty acid loss from the fractions of individual fatty acids lost for each repetition of the experiment greatly reduces the variability of the value

determined for the fraction lost of each individual fatty acid.

Data of total fatty acid masses presented in this paper leaves little doubt that fatty acid metabolism was affected by poisoning with pentachlorphenol. To determine whether or not KPCP poisoning had a selective effect on the metabolic processing of any individual fatty acid (i. e. whether under the added metabolic cost of poisoning some acids were preferred as substrates for the production of required energy and others were selectively conserved perhaps as requirements of structural integrity) can not be ascertained from the above data. One might suspect that a cell would regulate its machinery to preferentially destroy some acids and preferentially conserve others since it is stated that ω3 and ω6 acids are incapble of synthesis by fish and are required in the diet for cellular integrity (29). Some acids are found predominantly in the triglyceride fraction of cellular lipids presumably with little structural value and still other acids are found predominantly in the phospholipid fraction of cellular lipid where they are presumably an integral part of cell structure (26).

In general it was found that there was little selection for the retention or preferred destruction of individual fatty acids due to the increased cost of a faster metabolic rate induced by pentachlorophenol poisoning. The few acids which these data suggest may have been selectively retained with increased metabolic rate are not those which one might believe would be of the most value to the cell. The

data were handled in a way that would indicate selective destruction or retention assuming a simple method of fatty acid handling by the cell populations. The fact that in the large majority of cases no selection was found indicates that the assumptions made at least approximate the true situation in these experiments. These assumptions are 1. that the net rate of catabolism of any fatty acid is a function of its concentration and 2. that catabolic and anabolic processes are drawing from the same free fatty acid pool.

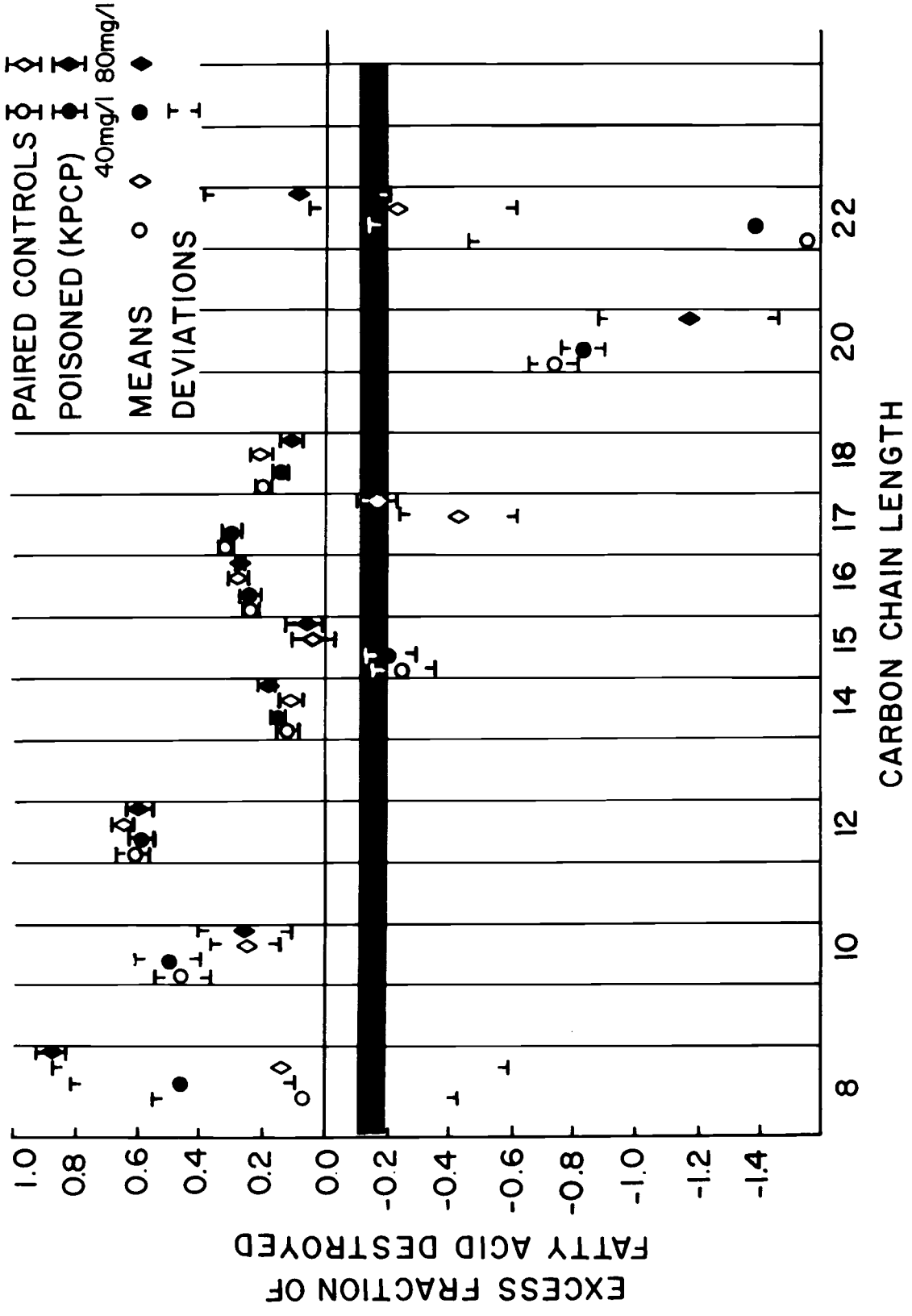
The net rates of catabolism or anabolism of individual fatty acids as measured in these experiments could be affected by a number of parameters. The metabolic rates of acids should depend upon the total metabolic rate of the cell., the concentration of the fatty acid, the concentration of enzymes affecting the fatty acid, the proportion of their contributions to the functional structure of the cells, and the total of the affinities of an acid for all of the enzymes affecting its metabolism. Other factors which would affect the rate of metabolism for a particular fatty acid such as temperature, pH and differing enzyme systems are thought to have been held reasonably constant by the nature of these experiments.

The amount of an individual fatty acid that was gained or lost to the closed system of these experiments over a ten day period was determined by subtracting the masses of that acid detected in the cell population and medium on day ten from the masses of that

acid detected in the cell population and medium on day zero. Assuming that the concentration of an acid affects its catabolic rate, the mass of each acid that was lost to the system was divided by the mass of that acid present on day zero whether in the cells or medium. The resulting fraction is the fraction of available acid which was lost over the 10 day period. Using as acid avilable only that in the cells or only that in the medium gave data in which no mathematically constant relationship was apparent. Analysis of the fractions of various fatty acids that were destroyed indicated that there was a rather constant relationship between the fraction of a particular fatty acid destroyed and the fraction of the total fatty acids destroyed in all of the control and poisoned groups of experiments. Subtraction of the fraction of total fatty acid destroyed from the fraction of each individual fatty acid destroyed not only gave similar resultant values between control and poisoned experiments but the resultant values also had in general smaller standard deviations than did the values of the fractions destroyed alone. The values on Figures 11 through 14 are presumably relative values that describe the relative tendencies each acid has for destruction. An examination of these values then may indicate the characteristics of acids that affect their rates of enzymatically induced change.

Figures 11 through 14 compare the tendencies for individual fatty acids to be destroyed. These comparisons are made between

Figure 11. Excess percentage destruction of individual saturated fatty acids above or below percentage destruction of total FAME by poisoned populations and by their paired control populations. Points below the stippled line represent acids which experienced net synthesis. Points above the line represent acids which experienced net destruction. Deviations indicated are standard errors.


PAIRED CONTROLS
POISONED (KPCP)
40mg/l 80mg/l
MEANS
DEVIATIONS
EXCESS FRACTION OF FATTY ACID DESTROYED
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
8
10
12
14
15
16
17
18
20
22
CARBON CHAIN LENGTH

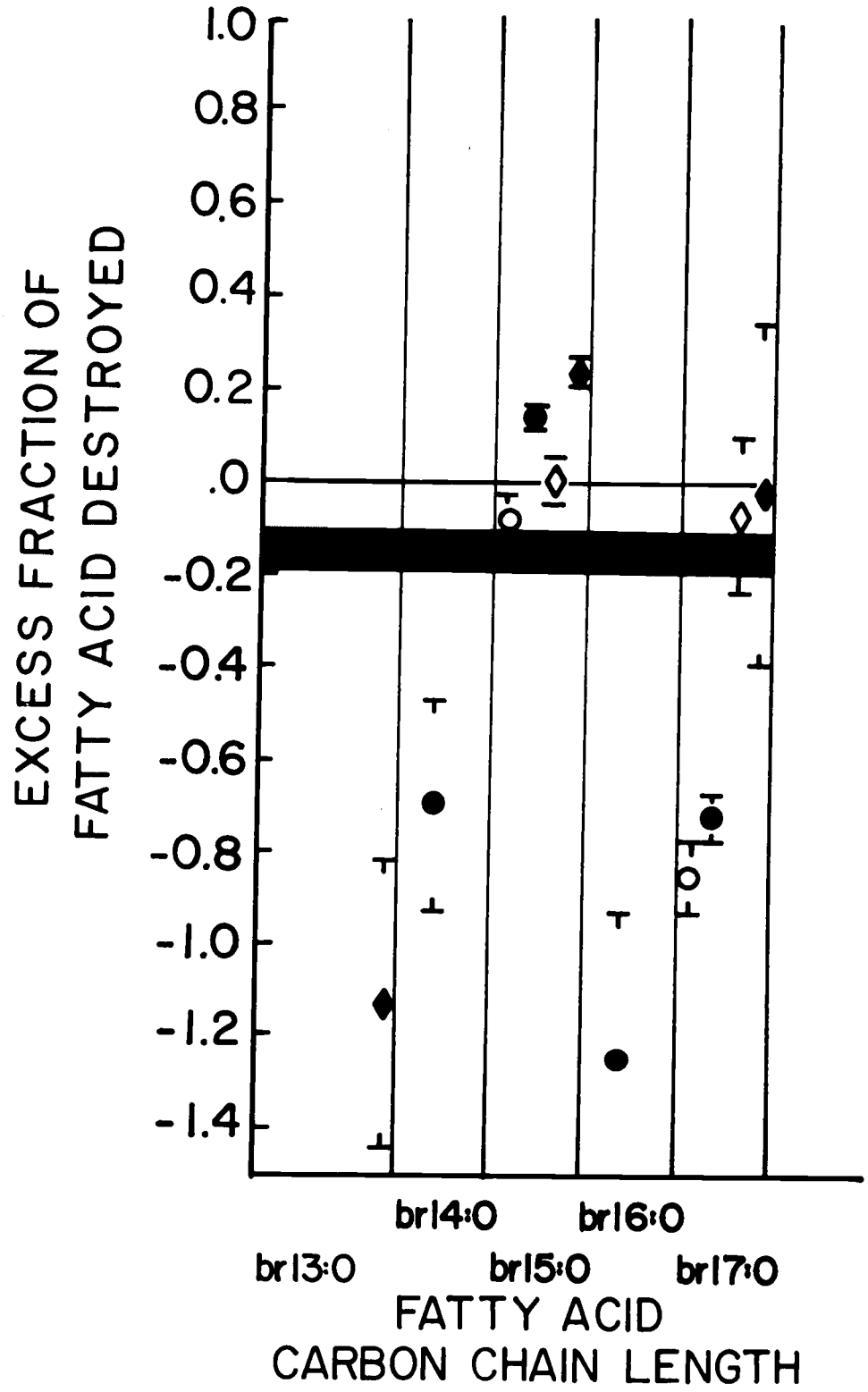


Figure 12. Excess percentage destruction of individual branch chain fatty acids above or below percentage destruction of total FAME. (See Figure 11 for legend.)

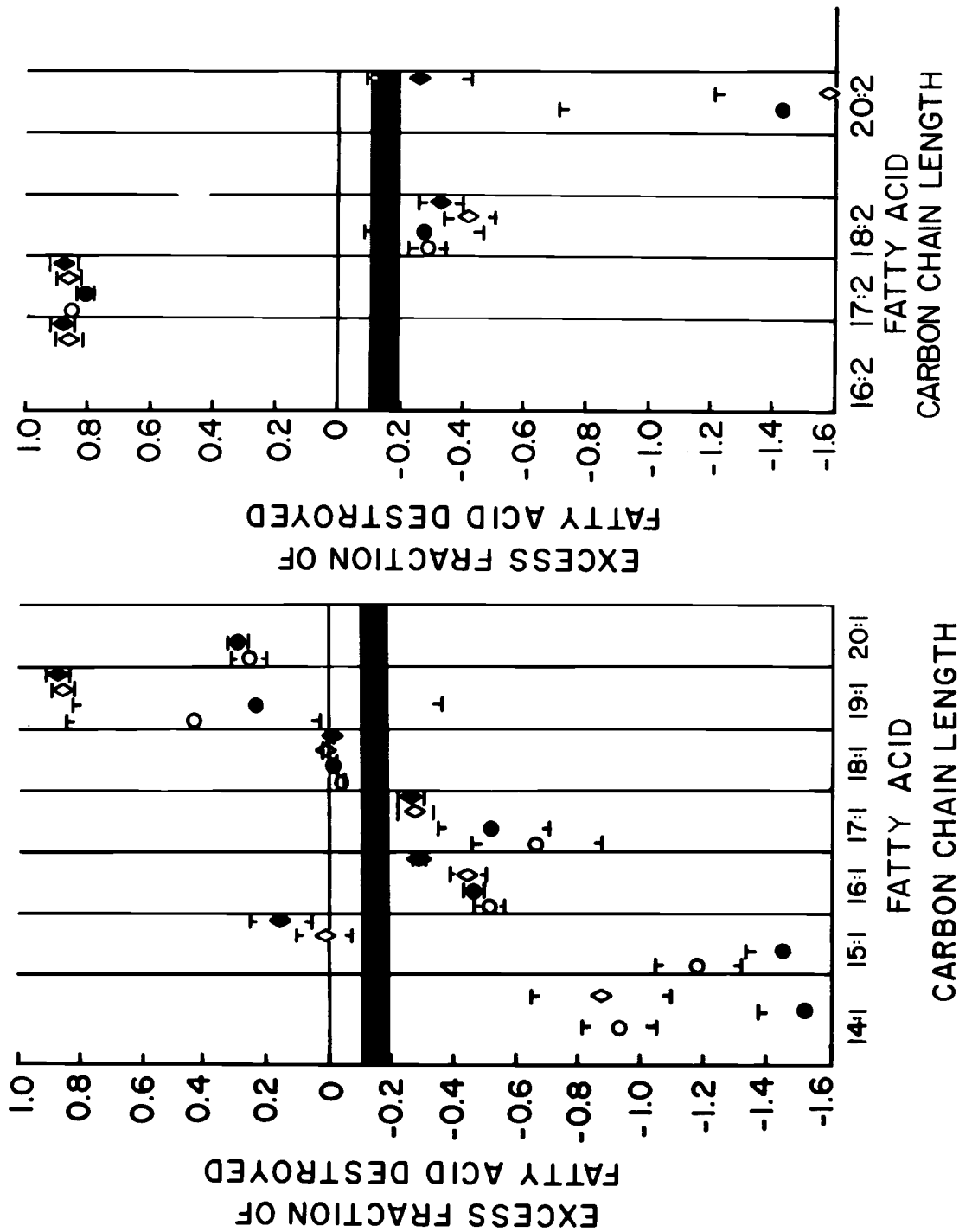


Figure 13. Excess percentage destruction of individual monene and diene fatty acids above or below percentage destruction of total FAME. (See Figure 11 for legend.)

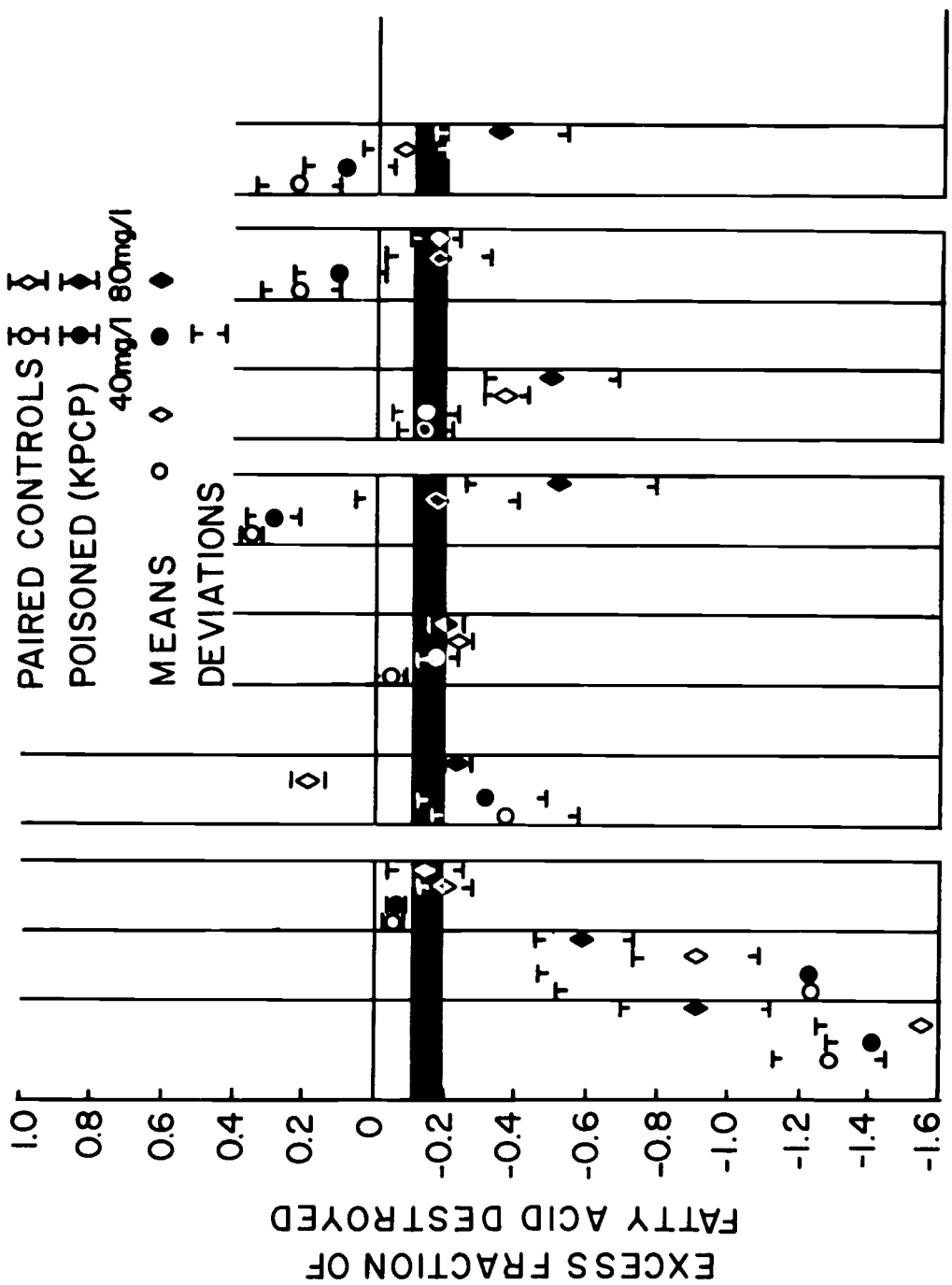


Figure 14. Excess percentage destruction of individual triene, tetraene, pentene and hexene fatty acids above or below percentage destruction of total FAME.

the different acids and between different experimental treatments on an equivalent molar basis and adjusted for total fatty acid catabolic differences between experimental treatments. The values in the figures are fractions of net catabolism of individual fatty acids minus the fractions of net catabolism of total fatty acid and the standard errors of these values. The resultant plot gives an indication of the excess or deficit fraction each fatty acid was catabolized compared to the total fraction of fatty acid catabolism and holds constant the effects that differing concentrations of each fatty acid would have on the rates of their catabolism. If a fraction on the graph is positive, the particular fatty acid thus represented has a higher than average tendency to be catabolized perhaps due to a better than average affinity for the catabolic enzymes or due to attachment to a complex lipid of higher than average turnover rate. If a fraction on the graph is negative, the particular fatty acid thus represented has a less than average tendency to be catabolized (comparative retention). A fatty acid which is synthesized to the point of net synthesis would be more negative on the graph than the fraction of total fatty acids lost in its particular set of experiments (below the stippled line). The average fraction of total fatty acids lost was 0.191 ± 0.077 for populations exposed to 40 mg KPCP/l and 0.151 ± 0.035 for populations of paired controls; 0.119 ± 0.113 for populations exposed to 80 mg KPCP/l and 0.139 ± 0.104 for populations of paired controls. A truly

selective change in the metabolism of an individual fatty acid due to a change in environmental conditions would be represented on the graph by one or more of the four points which represent a fatty acid being significantly different from the others. The two open points indicate control experiments and the two closed points, experiments involving KPCP. The open circle is the average from control experiments paired with six experiments involving 40 mg KPCP/1. The open diamond is the paired controls associated with the experiments involving 80 mg KPCP/1.

The horizontal zero line in these figures represents the average fraction of total FAME lost for each experimental treatment. Points in the figure represent increased (positive) or decreased (negative) fractions of individual fatty acids lost above or below the average fraction of total FAME lost. Points below the stippled area represent acids which have experienced net synthesis over the ten day period. Individual fatty acids are spaced along the abscissa of the graph relative to their carbon chain length. The deviations indicated are standard errors (standard deviations divided by the square root of the number of replications) unlike other deviations in this paper which are standard deviations for individual and not mean observations.

Control Populations

The paired control populations associated with the populations poisoned by 40 mg KPCP/1 will be discussed first. Then the paired control populations associated with populations poisoned by 80 mg KPCP/1 will be compared to the first mentioned control populations. Then will follow a discussion of the effects of poisoning on the tendencies for populations to selectively destroy fatty acids. For the convenience of discussion, the paired control populations to 40 mg KPCP/1 poisoned populations will be designated control populations A. The paired control populations to the 80 mg KPCP/1 poisoned populations will hereafter be designated control populations C.

Saturated Fatty Acids

The tendency for destruction of saturated fatty acids increased with chain length through acids 8:0, 10:0 and 12:0 (Figure 11). The tendency for destruction dropped sharply with chain length from acid 12:0 to 14:0 to 15:0. Acids 16:0, 17:0 and 18:0 were destroyed more intensely than was 15:0 but were similar to each other with a peak at 17:0. A sharp decrease occurs in the tendency for destruction from acid 18:0 to 20:0 and to 22:0. There appear to be two peaks in the tendency for saturated fatty acids to be destroyed with the greatest functional destructions occurring in acids 12:0 and 17:0.

The tendencies for destruction of the saturated fatty acids from control populations C (represented by open diamonds) appear to be significantly different from those discussed above for several acids although statistical pairing is not possible. Those acids with the greatest difference, between the two control populations, in their tendencies to be destroyed are acids 15:0, 17:0 and 20:0 and possibly 22:0. The value for 20:0 which does not fall on the graph is -2.56 with a standard error of 0.84.

Branch Chain Fatty Acids

The masses of branch chain acids were generally small so that the means in Figure 12 tend to have considerable variability. Values which did not fall on the graph and their standard errors are for control populations A: -4.73 ± 0.58(br13:0), -1.72 ± 0.22 (br14.0), -1.54 ± 0.43 (br16:0) and for control populations C: -4.86 ± 0.68 (br13:0), -5.23 ± 0.87 (br14:0), and -2.55 ± 0.60 (br16:0). Small masses and large variations make it futile to compare the tendencies for destruction among these acids.

Monene Fatty Acids

The value for acid 20:1 from control populations C and its standard error not appearing in Figure 13 is -1.62 ± 0.60. The tendencies for destruction of the monene fatty acids with an even

number of carbons in control populations A (represented by open circles) increase with chain length through acids 14:1, 16:1, 18:1 and 20:1. The tendencies for destruction of the monene acids with odd numbered chains also increase with chain length but the increase has a different slope indicating some difference in their handling by the cells than of the even numbered monene acids.

The tendencies for destruction of the even numbered monene acids from control populations C increase with chain length with a slope similar to those from control populations A with the exception of acid 20:1 which was synthesized and quite variable. The tendencies for destruction of odd numbered monene acids from control populations C were not consistent with chain length. Both acids 15:1 and 17:1 had increased tendencies for destruction and acid 19:1 was totally destroyed as none was found in day ten medium or populations.

Diene Fatty Acids

The values and standard errors for excess fractions of diene acids lost not appearing in Figure 13 for control populations A are: -3.69 ± 1.23 (16:2), -2.05 ± 0.45 (20:2) and for control populations C, -157 ± 0.35 (20:2). All of the diene acids with the exception of acid 18:2 occurred in small quantities so that the values are difficult to compare. Acid 17:2 was completely destroyed by all four population groups and acid 16:2 was completely destroyed by populations

exposed to 80 mg KPCP/1 and their paired controls.

Triene Fatty Acids

The triene acids are of special interest because they contain two isomers of an acid (Figure 14). These isomers (20:3 ω9 and 20:3ω6) in control populations A had different tendencies for destruction. There appear to be increased tendencies for destruction with increased chain length. There is little difference, between control populations A and control populations C, in the tendencies for triene acids to be destroyed.

Tetraene Fatty Acids

The tendencies for destruction of tetraene acids by control populations A (Figure 14) increased with chain length. The tendencies for destruction of these acids by control populations C seems nearly a reversal with a decreased tendency toward destruction with increasing chain length. The tendencies toward destruction of all of the tetraene acids are quite different in control populations A and control populations C.

Pentene Fatty Acids

The tendencies for destruction of pentene acids increased with chain length in both control populations A and in control populations

C (Figure 14). The two control groups differed in their tendencies to destroy pentene acids with control populations C having a greater tendency to conserve these acids.

Hexene Fatty Acid

The tendency to destroy acid 22:6 in the two groups of control populations differed, with control populations C having more of a tendency to conserve acid 22:6 (Figure 14).

Unknown Fatty Acids

The excess tendencies to destroy the unidentified fatty acids were -1.20 ± 0.19 (acid A), 0 ± 0 (acid B), 0.83 ± 0.02 (acid C), 0 ± 0 (acid D) and 0 ± 0 (acid E) in control populations A and -0.73 ± 0.59 (acid A), 0 ± 0 (acid B), -2.78 ± 1.06 (acid C), 0 ± 0 (acid D) and 0 ± 0 (acid E) in control populations C. Fatty acid A had a very strong tendency to be synthesized in both groups of control populations.

Degree of Unsaturation

The degree of unsaturation affects the tendency for fatty acids to be destroyed as well as the length of the carbon chain. The largest series of acids with the same carbon chain length but different degrees of unsaturation occur in the 18 carbon acids. A comparison of the

tendencies for these acids to be destroyed indicates a decrease in the tendency for destruction with increasing unsaturation through acids 18:0, 18:1, 18:2, and 18:3 in both control populations A and control populations C. The tendencies for acid 18:4 to be destroyed do not follow this trend, being only barely synthesized on a net basis in control populations A and being destroyed in control populations C. All of the acids in the two series (20:3 ω6, 20:4, 20:5 and 22:4, 22:5, 22:6) have similar tendencies to be destroyed in control populations A and control populations C with some trend toward more conservation of the more highly unsaturated acids.

Summary

Fatty acids in general have tendencies for destruction which are related to their degree of unsaturation and the length of the carbon chain. There were some differences in the tendencies for acids to be destroyed by control populations A and control populations C. The largest and most consistent differences were in a tendency for the polyunsaturated acids to be destroyed more by control populations A than by control populations C.

Poisoned Populations

Because of unavoidable and unknown differences in handling cell cultures the best comparisons can be made between pairs of control and poisoned populations. Only 12 fatty acids of the 40 studied had statistically significant differences in their tendencies to be destroyed between populations exposed to 40 mg KPCP/1 and their paired controls. Only 13 fatty acids had statistically significant differences in their tendencies to be destroyed between populations exposed to 80 mg KPCP/1 and their paired controls.

Saturated Fatty Acids

Two saturated fatty acids (18:0 and 20:0) had statistically significant differences in their tendencies to be destroyed between populations exposed to 40 mg KPCP/1 and their paired control populations (Figure 11). The probability of error in distinguishing the differences was less than 0.05 for both acids. Poisoning the populations with 40 mg KPCP/1 caused an increased tendency to conserve these acids.

Fatty acid 14:0 increased in its tendency to be lost with statistical significant in the 80 mg KPCP/1 poisoned populations ($p < 0.05$). Fatty acid 18:0 decreased in its tendency to be lost with statistical significance ($p < 0.001$) in the 80 mg KPCP/1 poisoned populations.

The direction of change from controls was the same as that in populations poisoned with 40 mg KPCP/1 for these two acids.

Branch Chain Fatty Acids

The value and standard error for the excess fraction lost for acid br13:0 from 40 mg KPCP/1 poisoned populations not appearing in Figure 12 is -2.33 ± 0.29. The values and standard errors for excess fractions lost for branch chain acids from 80 mg KPCP/1 poisoned populations not appearing in Figure 12 are: -4.12 ± 0.25 (br14:0) and -2.80 ± 1.27 (br16:0). Four of the five branch chain acids had different tendencies to be destroyed between populations exposed to 40 mg KPCP/1 and their paired controls. Acids br13:0, br14:0 and br15:0 were different with $p < 0.005$ and acid br17:0 was different with $p < 0.05$. Poisoning populations with 40 mg KPCP/1 caused increased tendencies for destruction of all of the branch chain acids.

Branch chain acids br13:0, br14:0 and br15:0 had increased tendencies to be destroyed with 80 mg KPCP/1 poisoning. The probability for error was less than 0.001 for each of these acids.

Monene Fatty Acids

Three monene acids, 14:1 ($p < 0.005$), 15:1 ($p < 0.01$) and 17:1 ($p < 0.05$), had statistically significant differences in their tendencies

to be destroyed between populations exposed to 40 mg KPCP/1 and their paired controls. Poisoning caused an increased tendency for the synthesis of acids 14:1 and 15:1 and a decreased tendency for the synthesis of acid 17:1 (Figure 13).

The values and standard errors for excess fractions lost for monene acids from 80 mg KPCP/1 poisoned populations not appearing in Figure 13 are -1.78 ± 0.35 (14:1) and -2.15 ± 0.71 (20:1). Three monene acids, 14:1 ($p < 0.01$), 16:1 ($p < 0.01$) and 18:1 ($p < 0.05$) had statistically significant differences in their tendencies to be destroyed between populations exposed to 80 mg KPCP/1 and paired control populations. The changes from paired controls were toward a decreased tendency to be destroyed for acids 14:1 and 18:1 and an increased tendency to be destroyed for acid 16:1. The changes in tendency to be destroyed due to poisoning for acids 14:1 and 16:1 were in the same direction in both 40 and 80 mg KPCP/1 poisoned populations but opposite for acid 18:1.

Diene Fatty Acids

All of the diene acids had very similar average tendencies to be destroyed in 40 mg KPCP/1 poisoned and paired control populations and none were significantly different due to poisoning (Figure 13). Acid 20:2 had a decreased tendency toward net synthesis due to 80 mg KPCP/1 poisoning with a probability for error

less than 0.05.

Triene Fatty Acids

Poisoning with 40 mg KPCP/l did not distrub the tendencies toward destruction of the triene acids (Figure 14). Poisoning populations with 80 mg KPCP/l caused significantly increased tendencies to be destroyed for acids 18:3 ($p < 0.001$) and 20:3 $\omega 9$ ($p < 0.05$).

Tetraene Fatty Acids

Acid 20:4 had an increased tendency to be conserved by 40 mg KPCP/l poisoned populations over paired controls with a probability for error less than 0.01 (Figure 14). Acid 18:4 had an increased tendency to be conserved by 80 mg KPCP/l poisoned populations ($p < 0.05$).

Pentene and Hexene Fatty Acids

Both acids 22:5 and 22:6 had increased tendencies to be conserved by 40 mg KPCP/l poisoned populations over paired control populations with a probability for error in concept of less than 0.5 (Figure 14). None of the tendencies of pentene acids and hexene acids to be destroyed were significantly different between populations poisoned with 80 mg KPCP/l and their paired controls. Changes due to 80 mg KPCP/l poisoning were generally an increased

tendency to conserve the pentene and hexene acids.

Unknown Fatty Acids

The values and standard errors of the excess fractions lost for unknown acids from 40 mg KPCP/l poisoned populations are: -1.16 ± 0.21 (acid A), 0 ± 0 (acid B), -0.85 ± 0.85 (acid C), 0 ± 0 (acid D), 0 ± 0 (acid E). Poisoning populations with 80 mg KPCP/l caused an increased tendency to destroy acid A over the tendency in paired control populations. The increased tendency toward destruction was statistically significant with a chance for error in concept less than 0.01. The direction of change was the same as that for poisoning with 40 mg KPCP/l.

Summary

Only a small proportion of the fatty acids studied had changed their tendencies for destruction under pentachlorophenol poisoning. Many changes that did take place due to poisoning were of minor extent. These data are interpreted as meaning that pentachlorophenol poisoning does not in general cause selective destruction or retention of individual fatty acids by altering the machinery of the cell which handles these acids. On the other hand, selective retention or destruction does take place due to pentachlorophenol poisoning. The selectivity, however, is a function of the relative tendencies

of each fatty acid to be destroyed and the average metabolic rate of the populations, which calls for more or less energy to be derived from fatty acid catabolism and more or less synthesis of fatty acids controlled by the energy necessary for these processes. The average metabolic rate of cell populations is disturbed by pentachlorophenol poisoning which secondarily disturbs the metabolic synthesis and/or catabolism of most fatty acids in the same proportion. Since different fatty acids are present in different quantities and also have different tendencies to be destroyed, the results of differing metabolic rates between populations are selectively increased catabolism of some acids and selectively increased synthesis of others.

SUMMARY

Indices of Poisoning

Embryonic cells from coho salmon grown in tissue culture tolerated 80 mg KPCP/1 over a 30 day (two cell passages) incubation period, but could not tolerate 120 mg KPCP/1 over this length of time. Growth of the cells was inhibited 50 percent by exposure to 66 mg KPCP/1 over a seven day period and the inhibition was a linear function of KPCP concentration. The embryonic cells in their medium were less susceptible to pentachlorophenol poisoning than were young coho salmon whose 24 hour LD_{50} was 0.15 mg KPCP/1 with death of all animals at this poison level within 96 hours (22). The reduced susceptibility of the cultured cells to pentachlorophenol poisoning could be due in part to the ability of albumins in the culture medium to bind pentachlorophenol (40).

Population Growth

Control cell populations increased 2.4 times the day zero value of their cell numbers over a ten day period. The average cell in control populations had a volume of 1873×10^{-12} ml and contained 194 pg of dry matter, 23.4 pg of matter after ashing, 28.7 pg of nitrogen, 21.0 pg of derived fatty acid methyl esters and 1717 pg of water.

Under the influence of 40 mg KPCP/1, the dry weight, fatty acid content and organic matter of cells after ten days of growth were reduced. At 80 mg KPCP/1, cell count, population volume, dry weight, ash, nitrogen, fatty acids and organic matter of the cell populations were reduced. Both control and poisoned cell populations removed fats from the media.

Forty mg KPCP/1 did not significantly reduce the number of cells in the day ten populations. The average cell exposed to 40 mg KPCP/1 had less volume, dry matter, ash residue, nitrogen and derived FAME; but none of these parameters were less than the values derived from paired control populations with statistical significance. While the cell count in populations exposed to 80 mg KPCP/1 was reduced, the average cell in these populations had more volume, dry matter, ash residue, nitrogen and total FAME than did the average cell from paired control populations. All of the increases in average cell parameters were statistically significant.

Fatty Acids

Thirty-five fatty acids were identified in control populations and five unidentified acids were encountered. Acids with one double bond constituted 48 percent of cell populations FAME and the predominant acid was acid 18:1 ω9 (39 percent). Saturated acids constituted 25 percent of total population FAME. The fatty acid compositions

of day zero medium, cell populations and day ten depleted media all differed indicating some selective handling of acids by the cell populations.

Control populations destroyed an average of 2.744 mg of FAME over the ten day growth period. Nearly all of this loss could be accounted for in lost saturated fatty acids. Net gains in mass occurred with several polyunsaturated fatty acids over the ten day growth period, with the greatest gain in 18:2ω9. Gains in mass were also noted in branch chain fatty acids.

Populations poisoned with 40 mg KPCP/1 destroyed an average of 3.650 mg FAME over the ten day growth period. The excess loss of FAME due to poisoning included increased losses of most saturated fatty acids and decreased gains of many polyunsaturated fatty acids.

Populations poisoned with 80 mg KPCP/1 destroyed an average of 2.313 mg FAME over the ten day growth period. The decreased loss of fatty acids by 80 mg KPCP/1 poisoned populations reflects the smaller number of cells in these populations. The average cell in the poisoned populations contained more fat than did experimentally paired control cells.

Selection of fatty acids by the cell populations for higher or lower than average destruction was found to be similar for specific fatty acids in all control and poisoned populations indicating that

pentachlorophenol poisoning, while causing increased total fatty acid catabolism by cultured cells did not generally induce a selective mechanism in the cells for the retention of fatty acids occurring primarily in structural fats nor did KPCP poisoning induce a selectively increased catabolism of individual fatty acids occurring primarily in depot fats.

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