Acetaminophen-Induced Hepatic Necrosis: A Reminiscence

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Running Title:
The Early Days: A Reminiscence

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Abstract

In the early '70s, Dr B. B. Brodie, Head of the LCP, NHI, NIH, initiated a program to elucidate the mechanism of hepatic necrosis induced in rats by bromobenzene. These studies showed a crucial role for its 3,4-epoxide intermediate, known in part, to collapse to 4-bromophenol. To examine a possible contribution of this phenol to tissue toxicity, some rats were co-administered a high dose of acetaminophen to suppress phenolic clearance by glucuronidation and sulfation. Subsequent examination of liver slices showed that the acetaminophen-only control rats had extensive centrlobular liver necrosis. This article is a personal reminiscence of the events that led up to this accidental observation, how it happened, and the subsequent resolution of the underlying mechanism, including the covalent binding of NAPQI to liver protein as the initial "hit", the glutathione protective threshold, the antidotal activity of cysteine, and the existence of the "therapeutic window" for antidotal therapy. Collectively, these studies formed the basis for antidotal therapy of acetaminophen overdose patients.

Significance Statement:
Studies in the early '70s extended Dr. B. Brodie's “Reactive Metabolites as a Cause of Tissue Injury” concept to explain the initial events leading to fulminant hepatic necrosis seen after overdoses of acetaminophen. This article, written by one of the key contributors, is a reminiscence of how the studies originated, how they were developed and their significance for therapy.
Biological research programs rarely, if ever, arise *de novo*. The acetaminophen hepatotoxicity mechanism program started with two serendipitous observations, with perhaps a third serendipity; that they happened in the right place, at the right time, with researchers of the right mixture of backgrounds.

The origin of the program can be traced to a presentation in 1967 by Dr. Bernard Brodie at a Ciba Foundation Symposium on Drug Responses in Man (Brodie, 1967). Dr. Brodie was Chief of the Laboratory of Chemical Pharmacology (LCP) at the (then) National Heart Institute. A current hot area of research was the role of the drug metabolizing enzymes in activating inert environmental chemicals, such as polycyclic hydrocarbons, nitrosamines, acetyl-aminofluorenes and azo dyes, to proximate or direct carcinogens (Miller and Miller, 1966). These reactive derivatives were shown to bind to cellular DNA, eliciting mutation during DNA replication. Brodies’ presentation, entitled “idiosyncrasy and Intolerance” included a section on adverse drug effects that cause structural and biochemical lesions, noting that the *in vivo* metabolism of many of such compounds give rise to acylating, arylating and alkylating agents, capable of forming
covalent links with tissue protein as well as with cellular DNA. He proposed that such covalent binding to tissue macromolecules could underlie many allergic responses, fetal abnormalities such as that caused by thalidomide, and direct tissue toxicity such as the lysis of red blood cells by aromatic amines like primaquine. As a harbinger of the acetaminophen story, the role of N-hydroxylation of the N-acetyl moiety of 2-acetylamidoflourene (2-AAF) was noted as an example of metabolic activation of amines to chemically reactive species (Weisberger and Weisberger, 1962).

To investigate this postulate, Brodie initiated a program at the LCP using bromobenzene as his model compound. Bromobenzene, a stable inert molecule, available commercially at high purity, had been demonstrated to cause hepatic necrosis in laboratory animals (Koch-Weser et al, 1952). The NIH site was well suited, having its own animal breeding facility such that rats were readily available at a day’s notice as well as histological and mass spectroscopy support. The LCP at that time had three research divisions, headed by Section Chiefs. Brodies’ program was assigned to the Section led by Watson Reid M.D., consisting of Gopal Krishna Ph.D, a Senior Staff Fellow, and two Fellows, Arthur Cho Ph.D., and Glenn Sipes Ph.D. James Gillette Ph.D., who headed the Section on Drug Metabolism & Pharmacokinetics, was closely involved in the work and later became Lab Chief and program director on Dr. Brodie’s retirement.

I arrived at the LCP in December of 1969, fresh from a post-doc under Dr. Daniel Ziegler at the University of Texas. Dan had isolated and characterized the first of the FMOs, (flavoprotein mono-oxidase) and shown that it metabolized secondary and tertiary amines including many antihistamines and other drugs (Ziegler et al, 1971). The relative
contribution of Ziegler's enzyme and the P-450 system for arylamine N-oxidation was still uncertain. Dr. Gillette kindly invited me to join his Section studying the mechanism of P450-dependent xenobiotic metabolism. My background was Pharmacy, followed by a M.Sc and Ph.D, in Biochemistry with a focus on cellular lipid metabolism and lipid structure/function. This experience of extracting lipids from tissue homogenates was to prove key for the later development of a reproducible covalent binding assay for both bromobenzene and acetaminophen.

The bromobenzene studies were well underway when I arrived and resulted in the report (Brodie et al., 1971) that bromobenzene and a number of other halobenzenes caused centrilobular necrosis in rats and that the severity of the lesion was massively exacerbated by pretreatment with phenobarbital to induce hepatic P-450. Autoradiograms of paraffin sections of mouse liver, 40 hours after a hepatotoxic dose of $^{14}$C-bromobenzene indicated that most of the radioactivity was found in the area of the lesion. Companion studies using rat liver microsomes, a dialyzed hepatic soluble fraction, a NADPH-generating system and $^{35}$S-labeled GSH, demonstrated conversion of many halobenzenes to their corresponding glutathione conjugates. Based on literature reports (Holtzman et al., 1967, Jerina et al., 1968, 1970, 1971) that glutathione conjugates, as well as phenols and dihydrodiol/catechol metabolites of halobenzenes arise through P-450 generated 3,4-epoxy intermediates, it was proposed that this electrophilic species could bind to nucleophilic sites on tissue macromolecules. This was the origin of the “reactive intermediate concept” as a cause of tissue lesions, including frank necrosis.
About this time and due to Dr. Brodies failing health, Jim Gillette took on increasing responsibility for the bromobenzene hepatotoxicity studies. I was reassigned to work directly under Dr. Brodie and shared a half-module lab with Jerry Mitchell M.D. Ph.D a newly arrived Pratt Fellow. Jerry’s project was polyaromatic hydrocarbon-induced bone marrow injury and mine was Brodies’ long-time interest, red cell injury by arylamines, using rat liver microsomal/red cell co-incubates. Neither project prospered, mine because the high endogenous lipid peroxidation in the controls obscured any arylamine-metabolite effect.

We were both reassigned, Jerry to work with Watson Reid’s group on species differences and effect of a non-specific inhibitor of P-450 enzymes (SKF-525A) on bromobenzene liver injury (Mitchell et al 1971), I to work with Gillette on the relationship between bromobenzene metabolism and hepatotoxicity. In addition, there was a need to explain an observation made by Watson Reid’s group (Reid et al., 1971), that pretreatment of rats with 3-methylcholanthrene (3-MC) to induce rat hepatic P-450 blocked the centrilobular necrosis caused by halobenzenes. Since the parallel enhancement of rat liver P-450 levels and hepatotoxicity was a major plank in the argument for the intermediacy of the 3,4-epoxide, and hence for the whole reactive metabolite concept, the observation that an alternate inducer of liver P-450 provided protection was a major complication.

Although the concept presented in Brodies’ 1971 paper, viz: that hepatic P-450 generates the electrophilic 3,4 epoxide, which subsequently binds to nucleophilic sites to tissue macromolecules and initiates liver cell death, seemed very reasonable and was widely accepted, there was little in
the way of direct supportive evidence. Similarly, that hepatic glutathione was protective was inherent in the concept but again, little direct evidence.

Nicola (Nick) Zampaglione Ph.D, a post-doc in Gillette’s Section joined me in the metabolism studies. Nick had graduated in Pharmacy in Italy and after migrating to the US, obtained his Ph.D in the laboratory of Gil Mannering in Michigan. Like Jim Gillette, Gil was a major player in the area of P-450 mechanism and function.

The metabolism studies were assigned the unglamorous role of tying up the loose ends, providing support for Brodies’ already widely accepted reactive metabolite concept of xenobiotic induced tissue toxicity. In practice, they turned out to be of greater import. Experimentally, using pulse iv administration of tracer doses of 14C-bromobenzene during the metabolism of a larger non-radioactive hepatoxic dose given ip in oil, they documented the threshold protection afforded by hepatic glutathione and that the metabolic fate of the 3,4-epoxide depended on cellular levels of glutathione: viz, initially by “non-toxic” conjugation in glutathione-replete cells followed by “toxic” covalent binding to cell protein when levels become depleted (Jollow et al.1974). The 3MC induction studies showed that this pretreatment, like phenobarbital, enhanced bromobenzene metabolism in vivo (albeit modestly) and that protection was associated with enhanced clearance via a 2,3-epoxide and epoxide hydrolase (Zampaglione et al., 1973). This principle, protection afforded by increase in competing non-toxic pathway(s) was to play an important role in the later acetaminophen studies.

Collectively, in addition to the crucial serendipitous experiment (discussed below) that opened up the acetaminophen hepatoxicity
mechanism study, this bromobenzene work provided the covalent binding assay, documentation of the glutathione threshold, and the key value of integrating pharmacokinetics, in vivo and in vitro metabolism and old-fashioned enzyme kinetics. As noted above, the 3MC studies made us aware that the dose-dependency of hepatotoxicity could be altered profoundly by change in non-toxic pathways of in vivo clearance without change in the activity of the toxic pathway.

Towards the end of the above metabolic studies on bromobenzene, Nick was reading papers from the laboratory of Jim and Betty Miller at the University of Wisconsin (Miller and Miller, 1966, Miller, 1970, DeBaun et al., 1970). It was known that the carcinogenicity of 2-AAF was mediated through N-oxidation to its hydroxamic acid, but that N-hydroxy-2-AAF had little direct carcinogenicity in skin tests. It was postulated that glucuronidation or sulfation of the N-hydroxy group was needed to enhance the leaving capacity of this hydroxyl group. To test the hypothesis, they co-administered 2-AAF to rats with and without a large dose of acetaminophen since it was well known that acetaminophen was largely cleared by these conjugation pathways (Brodie and Axelrod, 1948, 1949, Smith and Williams, 1949). We were aware that 4-bromophenol was a major metabolite of bromobenzene in vivo, that it arose by rearrangement of the 3,4-epoxide, and that it was cleared in turn, by conjugation to its glucuronide and sulfate urinary metabolites. Nick wondered if 4-bromophenol might contribute to the covalent binding and liver injury, and suggested that we test the idea by co-administration a high dose of acetaminophen with a minimal hepatotoxic dose of bromobenzene. I was very skeptical, being mentally locked into the 3,4-epoxide/glutathione
threshold mechanism. Nick’s enthusiasm won the day; rats were readily available, so why not? Ironically, as shown by later workers, 4-bromophenol can contribute to covalent binding to cellular protein, though apparently not to hepatotoxicity (Monks et al., 1982, reviewed Lau and Monks, 1988).

The rats were ordered, delivered and placed in a holding/experimental lab overnight. The next morning, they were divided into four groups: vehicle only, low hepatotoxic dose bromobenzene, bromobenzene plus acetaminophen, and acetaminophen alone. Twenty-four hours later, the animals were sacrificed and sections of their livers sent for preparation and staining with periodic acid-Schiff reagent and hematoxylin and eosin (PAS-H&E). Examination of the liver sections showed: vehicle only, normal liver, bromobenzene only, minimal centrilobular necrosis, bromobenzene plus acetaminophen, minor exacerbation, acetaminophen only, marked centrilobular necrosis. All of the rats in the acetaminophen-only group had liver lesions ranging from extensive to massive; what we would later rate as 2-3 plus lesions. It was unequivocally clear that we had an animal model for a human drug-induced fulminant hepatic necrosis (FHN), and at that, for a drug consumed by the millions. Unsurprisingly, this created a buzz in the LCP. It also ended our work on bromobenzene.

Jerry, who was not involved in the initial observation, went down to the NIH library and came back with the Boyd and Bereczky paper (1966), which had previously reported liver necrosis in rats given large doses of acetaminophen. Clearly our observation was not due to some experimental error. Subsequent literature review documented many recent British clinical
case reports of acetaminophen centrilobular liver necrosis after overdoses of the drug (Thompson and Prescott, 1966, McLean et al., 1968, Toghill et al., 1969, Proudfoot and Wright, 1970). Although in the clinical literature, it was not yet widely known and we were unaware of it.

Of importance, acetaminophen was excreted in part as a mercapturic acid metabolite, implicating a role for glutathione in its hepatic clearance (Jagenburg and Toczko, 1964). Thus, not only did we have an experimental model for a human drug-induced hepatotoxicity, the parallel with bromobenzene liver injury was striking; a similar centrilobular locus and a similar dependence on hepatic glutathione in metabolic clearance, implying in situ generation of a chemically reactive metabolite capable of forming covalent bonds with liver protein. We had an animal model, a probable mechanism, available animal and histological support, and experience in just this type of research, all in a laboratory setting ideal for this purpose. In essence, the bromobenzene studies had provided an experimental “blueprint” for exploration of the mechanism underlying acetaminophen FHN.

The fact that acetaminophen hepatotoxicity in patients was seen only after overdoses was reassuring, since human acetaminophen clearance via mercapturic acid was a very minor pathway, implying that pressure on the glutathione hepatic pool would be minor at therapeutic doses of the drug, but potentially overwhelming in the overdose situation. Hence the very large doses needed for the laboratory rats. All we had to do was to administer acetaminophen to rats and follow the bromobenzene “blueprint”.

Jerry joined the group, his clinical background and whole animal experience were to prove invaluable. Nick left the LCP to join the drug
metabolism group at Schering. We were joined by Don Davis PhD, a post-doc from Emory, and Bill Potter, a M.D Ph.D student from the University of Indiana. Later members include Snorri Thorgeirsson M.D Ph.D from the Hammersmith, and Dr. M. Hasimoto, on leave from Dainippon Pharmaceutical Co., Osaka Japan. The technical assistance of Ken Greene was also invaluable.

With high confidence, we repeated the rat study, acetaminophen vs vehicle control. Disaster, minimal or no discernable necrosis. Repetition, and there were many, gave the same result. The initial observation, backed up by the literature, was unequivocal. Clearly something was different in our experimental setup. One of the problems we were facing was the high glycogen levels in the livers which tended to obscure the, at best, minimal necrosis. As is well known, laboratory rats in a controlled light cycle lab tend to eat three times during the night, once when the lights go off, once in the middle of the night and once just before lights go on. To avoid the glycogen problem, we removed lab chow from the cages the night before experimentation. Success, these rats showed centrilobular necrosis, extensive to massive. We had our model back. This was the second serendipity, had we not goofed and forgotten to feed the rats overnight in the first experiment, it is likely that we would have missed the hepatotoxicity of acetaminophen. Our fed rats given even high doses of the drug showed little or no hepatic injury. We were accustomed to the unmistakable extensive lesions seen after bromobenzene and the very minimal lesions of fed rats probably would not have been recognized. Were it not for these two unrelated events, acetaminophen used as a metabolism modulator and accidental omission of food overnight, the acetaminophen hepatotoxicity-
mechanism program at the LCP, and its impact for molecular toxicology, would not have occurred.

Subsequent studies looking at the species difference in susceptibility indicated that hamsters and mice were most responsive, (fed) rats only at doses approaching the LD50, and guinea pigs and rabbits were refractive (Davis et al., 1974). Mice and (later) hamsters became the species of choice.

The first paper in the acetaminophen JPET series (Mitchell et al. 1973a) was analogous to the first bromobenzene paper, viz; the use of modulators of hepatic P450 to implicate reactive intermediate(s) formed during P-450-dependent metabolism as causative in the hepatic lesions. For both rats and mice, hepatotoxicity paralleled known P-450 modulation and was unrelated to tissue acetaminophen levels, clearly supporting the role of a P-450 mediated formation of a reactive metabolite. The mice presented something of a puzzle in that only piperonyl butoxide altered the half-life of the drug. While we had our suspicions, the explanation did not become clear until papers V and VI of the series (Potter et al., 1974, Jollow et al., 1974): viz, that at these doses, the half-life in the mice was determined by their rate of glucuronidation and not by their P-450 activity. Piperonyl butoxide suppressed glucuronyl transferase activity in both species and phenobarbital was inductive of this conjugative pathway only in the rat.

On submission to JPET, only this paper required more than minor editorial change. As for the bromobenzene study, the severity of the lesion had been presented as “minimal, extensive, massive”. One reviewer requested that this subjective assessment be replaced with the more
quantitative method of Chalkley (1943). This double-blind objective procedure involved extensive reassessment of the liver slides but significantly improved the quality of morphological assessment, and improved the assessment of the effects of species, dose and treatment on susceptibility of the animals to acetaminophen hepatotoxicity.

The second JPET paper dealt with the covalent binding reaction in vivo (Jollow et al., 1973) with a focus on the effect of the modulators of toxicity and the time dependence vis a vis acetaminophen clearance. The key observation was that the covalent binding reaction was greatest at a time when the bulk of the dose had already been eliminated. This set up the correlation with hepatic glutathione levels presented in paper IV. For a period of time this paper was much quoted, not for its brilliance but for the covalent binding assay. Better than being ignored.

Paper III took a classical biochemical approach to the covalent binding reaction, examining the dependence of rat and mouse liver microsomal activity on microsomal P-450. The focus was on the effect of treatments that modulated hepatotoxicity in vivo and on the species difference, rats vs mice. Several points are of interest. First, the covalent binding assay (Jollow et al., 1973), though reliable, was not “user friendly”, requiring multiple re-suspension of the protein in a “fluffy” state to facilitate extraction of non-bound radioactivity. The ability to generate the Lineweaver-Burk plot of Fig. 2, and the data of the Tables, speak highly of the meticulous work by Bill Potter with the assistance of Ken Greene. The Lineweaver-Burk plot clearly showed that the pretreatments of mice with phenobarbital and cobalt chloride had respectively, enhanced and suppressed the Vmax of the binding reaction without significant change in
the Km, confirming that the modulation of liver injury in mice seen in Paper I was associated with modulation of P-450. This paper was the first report of the ability of thiol agents, glutathione and cysteine, to suppress the acetaminophen toxic pathway. That this occurred in the absence of the 9,000g supernatant and hence of glutathione transferase, supported our belief that these agents acted as alternate nucleophiles. Notable in this paper, a direct comparison was made between the capacity of mouse liver microsomes to catalyze the covalent binding of acetaminophen and 2-AAF. Both reactions were enhanced by the addition of sodium fluoride in the reaction medium. This effect of fluoride on the microsomal N-hydroxylation of 2AAF and other arylamines was well known (Miller, 1970). As discussed below, these two observations were central to our overall concept of the mechanism of toxicity.

The fourth paper in the series, the ‘Protective Role of Glutathione’ (Mitchell et al., 1973b) described the time and concentration relationships between acetaminophen, hepatic glutathione depletion and covalent binding to liver protein in mice. These studies demonstrated that the binding and, by implication, liver injury occurs only after the glutathione protective capacity is lost. Further, that administration of cysteine, given before and after acetaminophen, protected the liver from both the covalent binding reaction and injury, foreshadowing the therapeutic use of N-acetylcysteine. In studies using hamsters, Papers V (Potter et al. 1974) and VI (Jollow et al., 1974) extended the correlation between necrosis, covalent binding, glutathione levels, and the pathways of metabolism of the drug. In addition to presenting the relevant data, these papers acted to bring
together the overall work as a unified concept, with the first publication of the diagram of proposed pathways vis a vis cell death (Potter et al., 1974).

These hamster studies confirmed that the effects of the various pretreatments on severity of liver necrosis need not correlate with their effects on the half-life of the drug. Pretreatment with phenobarbital enhanced glucuronidation, the major pathway of metabolic clearance, with little effect on the Vmax of the covalent binding reaction. Pretreatment with 3-MC enhanced the covalent binding Vmax but did not enhance glucuronidation or metabolic clearance. Compared with untreated hamsters, phenobarbital diminished and 3-MC enhanced liver injury. A similar disconnect between half-life and hepatotoxicity had been seen in the mice morphology studies of Paper I. Collectively, these observations illustrated the concept that for a drug such as acetaminophen, with a minor toxic and major non-toxic pathway, it is the ratio of the two activities that determine the proportion of the dose converted to the toxic metabolite and hence the severity of hepatic necrosis. This relationship may underlie the enhanced susceptibility to liver injury seen in chronic ethanol-induced/poor nutrition status patients after chronic high therapeutic doses of acetaminophen.

Looking back, it is interesting what did not appear in these papers. For example, we had shown that cysteamine inhibited the microsomal covalent binding reaction and protected against liver injury in vivo, but did not include these data in any of these papers. Since cysteamine lacks a carboxyl group, it cannot be converted to glutathione. We concluded that it had to be acting as an alternate nucleophile. However, lacking 35S-cysteamine we were unable to detect a cysteamine-acetaminophen
conjugate in *in vivo* and *in vitro* studies. This was troublesome but not surprising since such an adduct would have been present only at very low concentration. We assumed that this was a limit of detection problem. A better explanation would have to wait till the later work of others.

N-Acetylcysteine (NAC) as an antidote. Since Jerry and I had medical/pharmacy backgrounds respectively, we were well aware that NAC/Mucomyst® had been approved as a mucolytic agent in the early 60’s and that its wide commercial availability would make it the antidote of choice to prevent acetaminophen FHN after overdoses. Although discussed within the group and no doubt included in seminars, we did not mention it in any of these papers. Perhaps we thought that it was just so obvious that it didn’t need specific mention. In hindsight, a major omission.

The first schema linking metabolism to covalent binding was presented in papers V and VI (Potter et al., 1974, and Jollow et al., 1974b). In agreement with current evidence, it was assumed that the initial product of P-450 attack was the N-hydroxy derivative which subsequently dehydrated to the ultimate electrophilic quinone imine. The role of P-450 dependent N-hydroxylation of arylamides was well known from studies on in the hepatotoxicity and carcinogenicity of 2-AAF (Miller 1970). Our confidence was enhanced by our observation that the microsomal catalyzed covalent binding reaction of acetaminophen was enhanced by the inclusion of sodium fluoride in the medium, parallel to similar activation of 2-AAF (Potter et al 1973). Both reactions were decreased by depletion of P-450 by prior treatment of the mice with cobalt chloride. Microsomal metabolism of analogs, p-chlorocetanilide and p-ethoxyacetanilide (phenacetin) yielded stable N-hydroxy derivatives as the primary
metabolites (Hinson et al 1976, Hinson and Mitchell 1976). As shown by later workers, the first identifiable product of acetaminophen P-450 attack was the dehydration product, NAPQI. While mechanistically interesting, this did not change the overall concept; viz, P-450 activation to a reactive electrophile, detoxification by glutathione/glutathione transferase and, on failure of that mechanism, covalent binding to cell protein and cellular injury.

The first four papers in the JPET series, published as a block, were well received and had immediate significant impact. This was not surprising. While the bromobenzene studies had provided a “proof of concept”, the acetaminophen mechanism work had immediate clinical application. Acetaminophen’s use as an alternative to aspirin was growing and, as seen in Britain in the mid to late ‘60s, so would its overdose hepatotoxicity problem. These basic studies explained why acetaminophen was safe at therapeutic doses yet potentially life-threatening after an overdose, and provided a rational basis for the use of a widely available drug, N-acetylcysteine as an antidote. The glutathione threshold/covalent binding/liver injury relationship emphasized that therapy had to be initiated as early as possible, during the “window of opportunity”, to minimize the binding reaction and hence the initial “hit”. Looking back, two additional features also played a part. The first was the breadth of the study, ranging from morphometric analysis of liver necrosis, for which we can thank the anonymous reviewer, through pharmacokinetics and other whole animal studies to the in vitro biochemistry. Dr. Brodie had retired when these studies got underway and Jim Gillette was in charge of the program. Jerry and I were well aware that the quality of the work had to pass Jim’s high
standards. We were very appreciative of his mentoring and support, especially in the early days when control of the animal model was far from certain. The second was Jerry’s flair for salesmanship. It was Jerry’s idea to send the first four papers as a unit to the Pharmacology Society’s top journal. We were unaware whether this had ever been done and far from certain as to its reception. I believe that the resulting format, where the reader could go back and forth among the four papers, contributed significantly to their impact.

On a personal note, I joined the LCP as a visiting fellow and left four and a half years later as a Section Chief, Jerry and I being promoted for the acetaminophen studies. The LCP under Dr. Brodie and later Dr. Gillette had been a major force in the emergence of Pharmacology as an independent discipline. I had entered expecting well-equipped spacious labs with plentiful technical help. The reality was anything but, technicians were few and far between, equipment was adequate but in high demand. Permanent staff were in the minority, mostly post-doc and visiting fellows. Facilities were crowded, desks at the end of the corridor except when the Fire Marshall was due. What the place did have was activity, post-docs and visiting fellows trying to get as much out of their two years as possible. A wide variety of backgrounds, a place to learn other methodologies, other approaches to research. While there was competition, there was also cooperation. On arrival, I was assigned to share a half-lab with Henry Sasame PhD. a staff fellow working directly with Dr. Gillette. Henry kindly cleared three feet of desktop so that I would have a place to sit, adding the papers and journals to the pile already reaching a third of the way up the window. Henry would come in very early in the morning to prepare rat liver
microsomes before others needed the centrifuges. He, again kindly, offered to share microsomes so that I didn’t need to. It was not till sometime later that I realized that he would come in and prepare microsomes even on days he didn’t need them. Clearly, I have some very pleasant memories.

References


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Abbreviations:

2-AAF, 2-acetylamidoflourene, FMO, flavoprotein mono-oxidase, 3-MC, 3-methylcholanthrene, NAC, N-acetylcysteine, FHN, fulminant hepatic necrosis.