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Evidence for a Circadian Clock in the Fog Hypothalamus

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Abstract

All animals exhibit day/night rhythms in activity that follow a predictable pattern. These daily rhythms are controlled by pace makers, or circadian clocks. The circadian clock in mammals is located in the area of the brain called the suprachiasmatic nucleus (SCN). The SCN receives direct input from the eyes. This retinal input helps to keep the SCN circadian clock synchronized to the external photoperiod. When the SCN is removed from the brain and maintained under constant conditions, it continues to exhibit a 24 hour rhythm of activity.

While much is known about the mammalian SCN, the amphibian SCN has not been studied to as great a detail. Frogs exhibit strong circadian rhythms (e.g. in activity, calling, and mating) and are seasonally reproductive, but whether these rhythms are controlled by a circadian clock is unknown. The frog retina contains a circadian pacemaker and the pineal gland contains one as well. Whether an additional circadian clock resides in the frog's SCN has not been investigated. There is evidence that auditory input modulates amphibian daily rhythms. Furthermore, the frog SCN receives neuronal input from the brain auditory regions. Thus, there is a possibility that the frog SCN contains a circadian clock. Determining whether the frog SCN receives retinal input would be a good starting point for investigating whether the frog SCN does in fact function as an analog to the mammalian involved in circadian rhythm generation.

The area in the frog brain called the SCN has been defined solely on its being located directly dorsal to the optic chiasm. Whether it receives retinal innervation has not been studied extensively. Furthermore, its activity in vitro, i.e. whether it exhibits a circadian rhythm, has not been studied at all. My project involves tracing retinal projections in the hypothalamus of the frog, *Rana pipiens,* which will help to locate the SCN functionally. Once I have located the region I suspect to be the SCN, I hope to characterize its activity in vitro.

Introduction

All organisms exhibit day/night rhythms that follow predictable patterns. These daily, or circadian, rhythms are controlled by endogenous pacemakers, or circadian clocks. The primary circadian clock in mammals is located in an area of the brain called the suprachiasmatic nucleus (SCN), which is located immediately dorsal to the optic chiasm in the hypothalamus. The SCN receives direct input from the eyes which helps keep the SCN circadian clock synchronized to the external environment. When the SCN is removed from the brain and maintained under constant

conditions, it continues to exhibit 24 hr rhythms in neuronal and metabolic activity (Gillette, 1991).

The first study to investigate daily cycles was done using a plant that opened its petals during the day and closed them at night. It was hypothesized that this was a passive process driven by the perception of sunlight. However, Jean Jacques d'Ortous de Marian (1729) showed that in constant darkness plants continued to open and close their petals in a 24 hour cycle, refuting the original hypothesis. The word circadian, coined by Franz Halberg, describes the 24 hour nature of the rhythms. Circadian rhythms are self-sustaining oscillations produced by an endogenous pacemaker that continues to express itself without any external temporal information for 24 hours. (Ward, 1971)

The circadian clock is thought to have evolved to allow organisms to coordinate biologically important activities, such as feeding, sleeping, and mating. Cues from the environment, also called zeitgebers, (Mills, 1973), synchronize an organism's rhythms by a process of entrainment.

Light, through the period of daylight, is the strongest environmental cue to entrain circadian rhythms. By its very nature, it is daily and seasonal; therefore serves as a zeitgeber. (Hartwig, 1982) Not only does it give information about the time of day, the length of daylight can give vital information about the season of the year. Organisms kept in constant light conditions will exhibit oscillations that run on a 24 or 26 hour rhythm. The environment insures these cycles are on a regular 24 hour schedule. (Ward, 1971)

A commonality among all the environmental cues is that they are be perceived by some sensory organ. It can be postulated that information from these sensory organs project in part to a circadian rhythm generator responsible for coordinating the activity of other biological processes. This center would be the first step in a chain of events that conveys temporal information to other systems of the organism. (Florez, et al, 1995)

There are three regions of the vertebrate brain which have been shown to contain circadian clocks. The first is the eye. The retina has this role largely because it is directly photosensitive. The mechanism of how it is able to generate rhythms is unknown. Yet it is known that the retina is a significant contributor to the circadian system in all vertebrates, including mammals. (Menaker, 1982)

The second region is the pineal gland. Like the retina, the exact mechanism by which the pineal gland acts to generate rhythms is unknown; however, it is believed to be involved in modulation of melatonin levels. In non-mammalian species, it is directly photosensitive, allowing it to be able to detect diffuse changes in light intensity. (Delgado, 1989) Interestingly, it is not able to generate circadian rhythms in mammals. (Hartwig, 1982)

The final area is the one that has been studied extensively in mammals. This is the suprachiasmatic nucleus (SCN) of the hypothalamus. In all cases where it is known to have a circadian clock, it also receives retinal projections. It is through this pathway that the clock receives information about the external photoperiod. (Moore-Ede, et al, 1982)

Reptiles and birds have circadian clocks located in their retinas, pineal gland, and hypothalamus. Thus, their circadian systems are extremely redundant. Mammals, on the other hand, have no circadian clock in their pineal gland, and a circadian clock has only recently been discovered in the retina. Thus, their circadian systems are less redundant. (Figure 1; Hartwig, 1982)

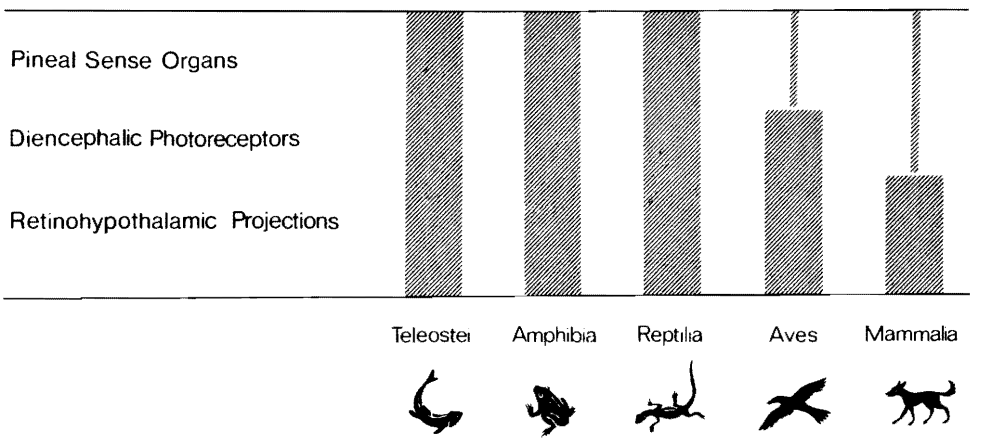

Figure 1: Comparative survey indicating the presence of (1) pineal sense organs, (2) diencephalic photoreceptors, and (30 retinohypothalamic projections in the vertebrate phylum. *Hatched columns* presence of photosensory system indicated on the left side of the diagram. *Hatched line* photosensory capacity not shown in threshold experiments. Only limited numbers of species have been investigated *(Taken from Hartwig, 1982)*

Frogs exhibit strong circadian rhythms (e.g., in activity, calling, and mating), but the organization of their circadian system has not been studied extensively. Both the frog retina and

pineal gland contain circadian pacemakers that generate rhythms in melatonin secretion and entrain to the external light cycle. (Skene, et al, 1991; Cahill, et al, 1991) Whether an additional circadian clock resides in the SCN has not been investigated. However, there is evidence that auditory input modulates amphibian daily rhythms, and the frog SCN receives neuronal input from brain auditory regions (Wilcynski, 1993). These data suggest that the frog SCN might contain a circadian clock. It could be hypothesized that the frog SCN may be acting as a circadian rhythm generator that is receiving some sensory input from the auditory system.

The area in the frog brain designated the SCN has been defined solely on its being located directly dorsal to the optic chiasm. Only one study, using the green treefrog (*Hyla cinera*), has demonstrated a retinal input to this area (Wilcynski, 1993). Determining whether this projection is found in other frogs could strengthen the theory that the frog SCN contains a circadian clock because it would show similarity between the frog SCN and the SCN of all other species which contain circadian clocks.

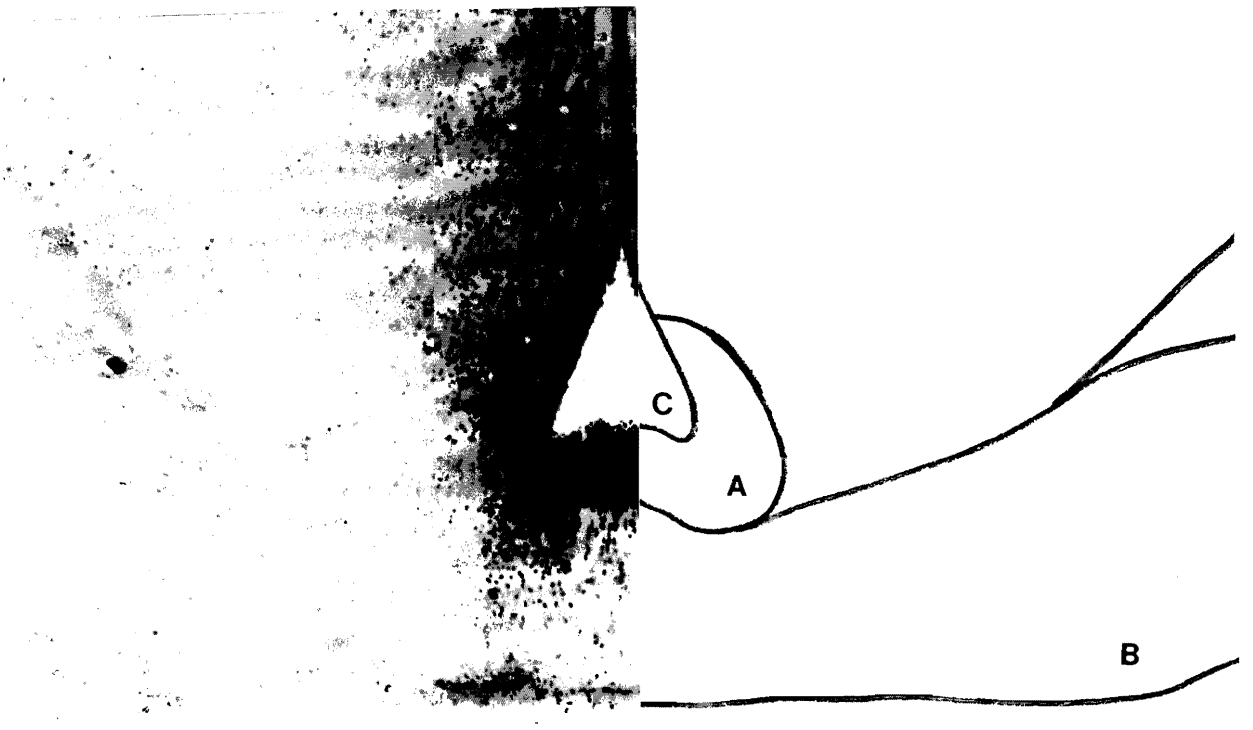

Figure 2: Neutral Red staining reveals the cell somata of the region. The outline sketch shows the SCN with cell bodies that are more tightly packed than the surrounding regions. SCN (A), Optic Chiasm (B), Third Ventricle (C). *Jim Hall*

Furthermore, determining its activity *in vitro*, i.e., whether it exhibits a circadian rhythm, will be critical to determining whether the frog SCN contains a circadian clock. To be able to generate a circadian rhythm, the structure must exhibit activity without any external stimuli. When all of the cells around the circadian clock region no longer receive stimuli, they will be metabolically inactive. In contrast, the circadian clock tissue will still be active. One way to test for *in vitro* activity is to incubate the tissue in methylene blue, a dye which is selectively taken up by metabolizing cells. Figure 3 shows an example of such a test in rat SCN tissue.

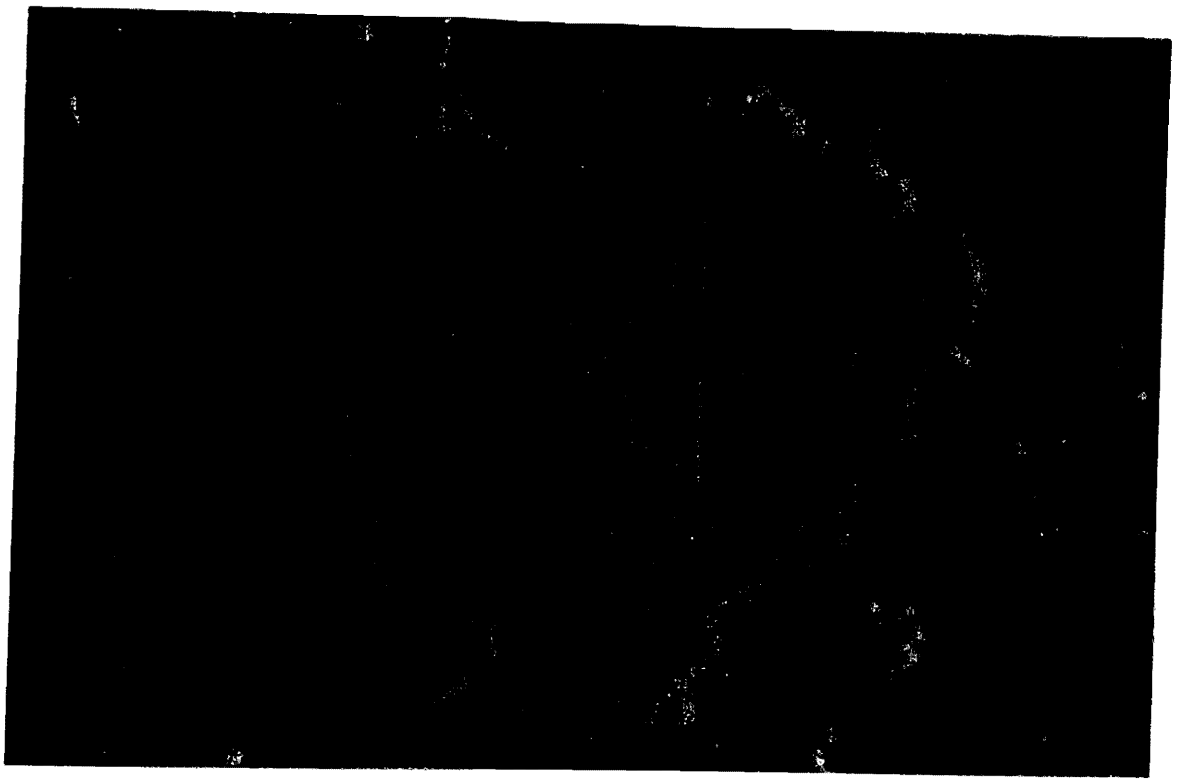

Figure 3: Methylene Blue stains the SCN of the Rat Hypothalamus. The arrowheads point to the stained SCN. In the outline sketch, SCN (A), Optic Chiasm (B). *Rebecca Prosser*

The goals of this study were twofold:

(1) To determine whether the hypothalamus of *Rana pipiens* receives a direct retinal projection, and

(2) To determine if this same area of the hypothalamus exhibits higher levels of metabolic activity in vitro than surrounding areas.

With this information, it will be possible to conduct electrophysiological recordings in the identified area to search for rhythmic neuronal activity.

Materials and Methods

Methylene Blue Staining

500 μm coronal brain slices containing the SCN and optic chiasm were made from adult *Rana pipiens* frogs using a McIlwain tissue chopper. The slices were maintained in a brain slice chamber, constantly perfused with frog Ringers (110 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl2, and 2.4 mM NaHCO3) gassed with 95%O2/5%CO2, for 1-2 hrs. The slices were then incubated for 1 hr in 15 ml of frog Ringers to which 2 drops of saturated methylene blue solution were added. After the incubation, the slices were washed three times for ten minutes with frog Ringers, and then refrigerated.

Horseradish Peroxidase Injection

Intraocular injections of 15-25 μL of 15% horseradish peroxidase (HRP) were made in adult leopard frogs, *Rana pipiens*, under tricaine methylsulfonate (MS 22) anesthesia. HRP is taken up by retinal ganglion cells and transported anterogradely to their terminals. Injections were made using a Hamilton microsyringe that was left in the eye for a short period before removing to reduce the loss of HRP. The frog was then allowed to recover. After 2-3 days, the frog was anesthetized by immersion in 1% MS22 then transcardially perfused with cold concentrated saline followed by cold 1 M phosphate buffered 4% gluteraldehyde. The brain was removed, post-fixed in 1 M phosphate buffered 4% gluteraldehyde for 2 to 4 hours, and embedded in a gluteraldehyde fixed block of egg yolk. Serial 50 μm slices through the hypothalamus were made on a Campden Instruments Ltd vibrating microtome. For the DAB reactions, slices were washed in 1 M PBS, Tris HCL buffer, then 2 minutes in 0.5% Cobalt Chloride in Tris HCL buffer. After washes with Tris HCL buffer and PBS, the slices were preincubated for five minutes in 48 mL PBS and 30 mg DAB in 2 mL H2O. Then 0.7 mL of 1.5% hydrogen peroxide was added. This was left incubating for 15-20 minutes. The slices were then washed with PBS.

After drying overnight, the sections were rinsed in PBS, dehydrated with an ascending (70-100%) series of alcohol baths, cleared with two xylene baths, and then coverslipped with Permount.

Results

Staining the frog hypothalamus with methylene blue: 11 frogs were used, with 2 to 3 slices of hypothalamus in each frog moving laterally through the SCN. 4 frogs showed the same pattern of staining in the SCN which can be seen in figure 3. The methylene blue became trapped in the optic chiasm in all of the 11 frogs, causing it to stain a dark blue. This made distinguishing the SCN from the optic chiasm difficult. However, on close observation, the ventral SCN appeared stained by the methylene blue.

Figure 3: Methylene Blue stains the ventral SCN of the frog hypothalamus. In the outline sketch, the ventral SCN (A) is the region directly above the optic chiasm (B). Third ventricle (C).

Injection of HRP into the frog eye: 5 frogs were injected with HRP. Upon analysis of the tissue, only 1 showed HRP penetration through the optic chiasm. This single trial showed a bundle of fibers projecting from the retina. These fibers could be seen crossing at the optic chiasm. A bundle of these fibers did penetrate into the SCN. They appear to end abruptly at the ventral margins of the SCN. They do not penetrate deeply into the entire SCN.

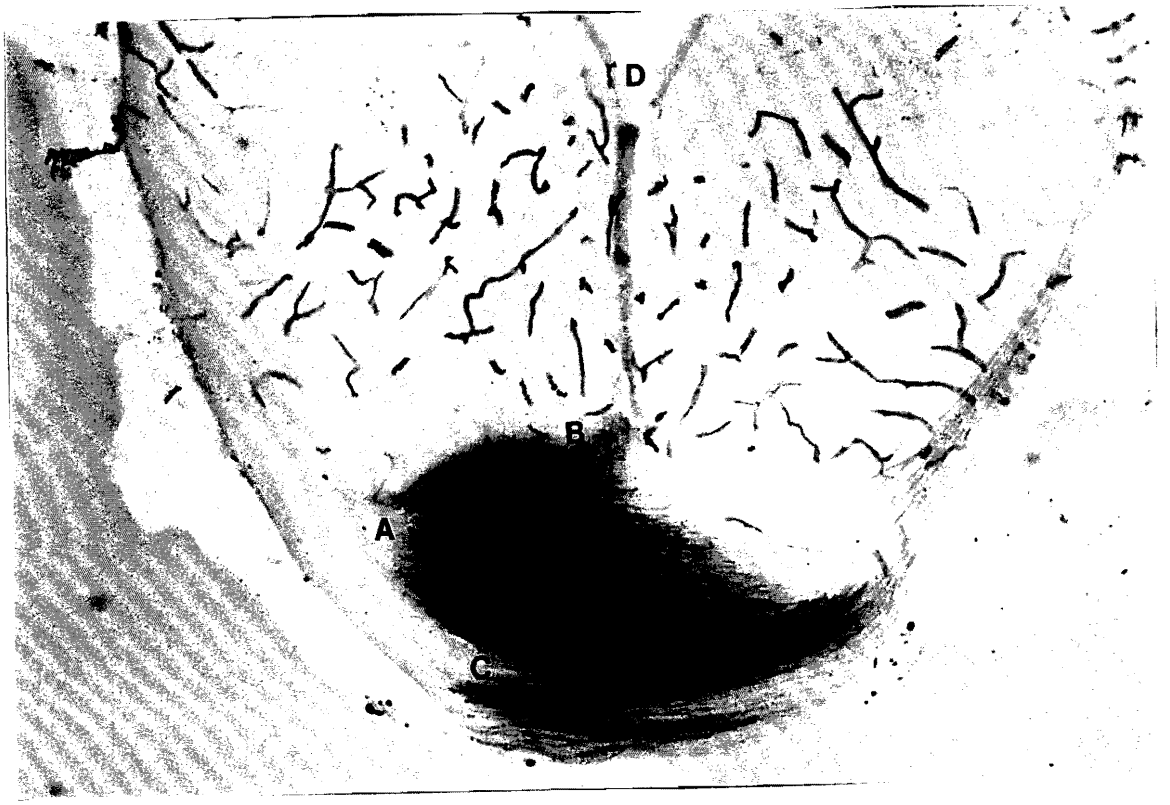

Figure 4: HRP staining reveals a retinal projection into the hypothalamus. The fibers (A) are stained dark brown. A bundle of fibers project up and out of the optic chiasm into the ventral region of the SCN (B). Optic chiasm (C), Third Ventricle (D).

Figure 5: Higher magnification shows more clearly that the fibers stained with HRP project into and terminate in the ventral portion of the SCN.

Conclusions

From the evidence presented, two main conclusions can be drawn. First, the HRP injections indicate that there are retinal projections to the ventral margins of the SCN of the frog species *Rana pipiens*. By having these projections from the retina, the frog SCN has a characteristic common to all circadian rhythm generating SCN. While alone it does not qualify the frog SCN as a circadian clock, it is an important detail.

Second, the methylene blue staining suggests that the ventral SCN exhibits higher metabolic activity than surrounding hypothalamic structures during the daytime. This evidence is important because it shows another characteristic that the frog SCN has in common with SCN in

other organisms that contain a circadian clock. In addition, it shows that the frog SCN can be active independent of external stimuli. This *in vitro* metabolic activity suggests that the frog SCN could be a circadian rhythm generator.

Taken together, these results point to the ventral SCN as a possible location for a circadian clock in the frog. It would be important to repeat many parts of this project to guarantee the results. Also, in both protocols, future studies could utilize different methods. For example, the HRP injections could be tried with fluorescent dyes such as FITC or Luciferase compounds. It may even be worthwhile to look for dyes which remain in the animal longer, compounds which would have a longer time to diffuse through the neurons. In the methylene blue incubations, different compounds could be used to detect metabolic activity. For example, radiolabeled metabolites, such as di-deoxyglucose could be added to the media. Also, it may be possible to use the information about the genetics of circadian rhythms to try to locate frog homologues of genes such as *per* in the frog SCN tissue.

Acknowledgements

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Nuclear Regulatory Policy

When the Cold War began in the mid 1940's, the United States paid much more attention to getting ahead in the nuclear arms race than it did in exploring the environmental repercussions of doing so. Gradually, scientists and the public began expressing concerns about the safe storage of spent nuclear fuel and the byproducts of nuclear power and weapons production. What followed was a series of Congressional regulations that became a bit more stringent with each new promulgation. This gradual trend gave technology the opportunity to advance (cleanup methods) and also allowed the enormous cost of cleanup to be spread out over a longer period of time.

The Atomic Energy Act of 1946 was the first Congressional regulation dealing with atomic energy. This statute established the Atomic Energy Commission (AEC), which was to conduct research and development on the peaceful applications of fissionable and radioactive materials.

The Atomic Energy Act of 1954 stressed domestic and international uses of the atom and also provided for the control of source material and by-product material (radioactive substances). It is the primary source of federal authority for regulating nuclear materials. In *Northern States Power Co. v. Minnesota*, 405 US 1035 (1972), the Supreme Court decided that the federal government has the right to regulate control and operation of nuclear materials, including disposal of nuclear wastes (Herzik 54).

The Price-Anderson Act originally passed in 1957 amended the Atomic Energy Act by encouraging financial responsibility of nuclear plant owners. Plant owners who obtain sufficient liability insurance according to the act's guidelines were shielded from unlimited tort recoveries. The Act was amended in 1966 to prohibit participating nuclear

entities from assessing legal defenses of governmental immunity and contributory negligence. Further Amendments in 1988 limited liability to $560 million in the event of an extraordinary nuclear occurrence. Punitive damages cannot be awarded for a nuclear incident or precautionary evacuation. The Price-Anderson Act does not clearly provide the same liability dollar limit protection to transporters and storers of radioactive wastes as it does to nuclear producers. Therefore, all transporters and storers of nuclear waste obtain insurance which will be able to cover all costs of an accidental spill (because of high risk, this is very expensive). Insurance companies will rarely insure against claims for punitive damages, civil fines and penalties (Herzik 55).

The National Environmental Policy Act of 1969 (NEPA) stated its purpose as, "to prevent or eliminate damage to the environment and biosphere and stimulate the health and welfare of man." (Murray 159). NEPA created the Council of Environmental Quality (CEQ), an advisory group reporting to the President. This Act also established the Environmental Impact Statement, which must accompany any federal action that may significantly affect the environment. An EIS is a large document that describes alternatives, potential environmental, economic, and social effects, includes public comments and agency answers and reports the findings of hearing boards. The Environmental Protection Agency was also created to regulate air and water standards, establish limits on pollution and control radioactive materials. The EPA provides for public participation through meetings, hearings and advisory group reviews (Murray 159). The Energy Reorganization Act of 1974 divided the jurisdiction of the Atomic Energy Commission between two agencies, the Energy Research and Development Administration

(ERDA) and the Nuclear Regulatory Commission (NRC). The Energy Reorganization Act of 1977 replaced the ERDA (Murray 159).

The Nuclear Regulatory Commission has jurisdiction over reactor construction and operation. It also licenses and regulates the possession, use, transportation, handling and disposal of radioactive materials. Agreement states accept authority to control radioactive wastes under NRC guidelines. Title 10 Energy, contains 1400 pages of regulations on radiation standards and reactors (Murray 160).

The Department of Transportation (DOT) provides rules on the transportation of radioactive materials, and the Federal Emergency Management Agency (FEMA) prepares plans for emergency response to radioactive releases.

In 1983, the Department of Energy issued the Defense Waste Management Plan. This plan called for the construction of waste treatment and disposal facilities, the construction of new storage facilities, and a safer transportation system for nuclear wasted (Herzik 128). It was produced in response to Congressional pressures for the Department of Energy to provide a direction for nuclear waste cleanup.

The U.S. Environmental Protection Agency has been charged with enforcing the clean-up provisions of the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA) and its amendments contained in the Superfund Amendment and Reauthorization Act of 1986. Should a nuclear spill occur during transportation, the EPA and its state counterpart will spearhead the cleanup effort (Herzik 56).

In 1988, Congress included language about environmental contamination in the Defense Authorization Act. In 1989, Department of Energy (DOE) Secretary, James

Watkins, stated that environmental protection and assurance of safety and health took precedence over production. "Tiger Teams", groups of DOE and contractor experts were sent to inspect facilities across the nation. These teams assessed compliance with rules and laws and prepared reports for corrective action. The teams inspected 35 major facilities and issued 8715 findings. This study led to the establishment of an Environmental Safety and Health Progress Assessment Program to implement findings (Murray 142).

A key conclusion of the study was that safety concerns existed at many of the facilities. A total of 111 U.S. inactive sites were identified and a date of 2019 was set as a goal date for cleanup. To accomplish the time frame, DOE created the Office of Environmental Restoration and Waste Management (EM). Many issues and challenges face this program: 1) Coordination with the EPA Superfund program (EPA has identified 25 radioactively contaminated sites, along with sites with soil contamination due to natural radionuclides); 2) What degree cleanup is feasible (can't make totally clean for technological, economic and social reasons); 3) The need for new technologies (robotics and new separation technologies); 4) Requirements for environmentally trained personnel (takes time and money to retrain employees in nuclear cleanup field); 5) Must convince the public that this expensive project with little visible signs of a product deserves support and must be done; 6) Establishing priorities for cleanup (most of the content is unknown, and studies must be performed to see which areas are in need for quickest cleanup; 7) Deciding on the applicability of the concepts of ALARA (as low as reasonably achievable) and BRC (below regulatory concern); 8) Finding sites for disposal facilities that will accommodate decontamination and decommissioning waste; 9) Need to manage

effectively (comprehensive management system is needed for entire nationwide process). (Murray 143)

Concerns about the possibility of having no place to dispose of low-level radioactive wastes led the states to seek control of waste management. This led to the Low-Level Radioactive Waste Policy Act of 1980. The legislation says, "Each state is responsible for providing for the availability of capacity either within or outside the state for disposal of low-level radioactive waste generated within its borders (excluding defense or other federal wastes). Low-level radioactive waste can be most safely and effectively managed on a regional basis." (Murray 160) This act created compacts among several states. Compacts decide what facilities are needed and which state will serve as the host and for how long.

Then, in 1985 Congress passed the Low-Level Radioactive Waste Policy Amendments Act. It called for keeping three commercial disposal sites open through 1992 due to the longer-than-anticipated time necessary for the states to form compacts. It also set volume limits on the wastes that could be sent to these sites. The Act called for deadlines on ratifying compacts, selecting host states, developing plans, submitting license applications and providing for disposal. The Department of Energy was able to allocate additional storage capacity to reactors in cases of emergency, and the Nuclear Regulatory Commission could authorize emergency access to the existing sites (Murray 161).

Establishing low-level waste storage facilities by the deadline of 1996 has been slow for a number of reasons: 1) each project must develop its own selection process for a disposal site; 2) a survey of a complete host state for potential sites involves the collection and analysis of enormous amounts of data; 3) the processes of site

characterization and interpretation of data are long and involved; 4) the application for a license is a many volume document; 5) the regulatory review of the application is thorough and extensive; and 6) lawsuits initiated by potential host communities or intervening groups delay action (Murray, 162). This lack of storage facilities has added great expense to generators who now have to store wastes on site.

In 1982, Congress passed the Nuclear Waste Policy Act for the management of high-level radioactive waste and spent fuel. It contained input from industry, government, and environmentalists and a timetable for action by the Department of Energy leading to underground disposal of high-level waste.

The Act was designed to regulate primarily commercial waste materials, but provides for defense waste disposal upon Presidential approval. A nuclear waste fund was setup to pay for disposal, with money coming from the waste generators themselves, who in turn charge their electricity customers. The fee was established at 1/10 cent/kilowatt hour (Murray, 162).

Following passage of the law, DOE established the Office of Civilian Radioactive Waste Management (OCRWM). Guidelines were issued for the process to select suitable sites for a repository, a Mission Plan was developed, and geological surveys were begun. Nine sites were identified as potentially acceptable, and this number was further reduced to three sites in 1987: Hanford, Washington, Yucca Mountain, Nevada and Deaf Smith County, Texas (Murray, 163). After the passage of the Nuclear Waste Policy Amendments Act of 1987, Yucca Mountain would be the only site characterized.

It was decided to use a Monitored Retrievable Storage (MRS) system, and studies were conducted on how to best implement it. A limit on storage capacity of the facility

was set at 10,000 tons. A license must be granted by the Nuclear Regulatory Commission before the MRS can be built. This is the legal device that prevents the MRS from becoming a permanent storage facility (Murray 163).

The 1987 Act added a number of special features: A Nuclear Waste Review Board in the National Academy of Sciences was created; spent fuel must be shipped in NRC-approved packages, with state and local authorities notified of the shipments; authority was given for continued studies of the sub-sea-bed disposal option; no further crystalline rock studies were allowed; and DOE is to submit a study for the needs of a second repository in the period from 2007 to 2010 (Murray 163).

Progress in characterization of the Yucca Mountain site have been slow due to the efforts of the State of Nevada to halt the project. DOE finally obtained approval to proceed and not be held up by permit requirements. The main concern expressed was regarding pathways on the premises that would allow rapid radionuclide transfer on the site.

A timetable was revised to accept spent fuel from utilities by 1998 and begin waste disposal in 2010. To accept the fuel, DOE must have the Monitored Retrieval Storage facility ready. This in turn depends on the success of the OCRWM to characterized the site efficiently and to avoid legal obstruction. Finally, adequate funding must continue if this project is to reach fruition (Murray 164).

In 1992, Congress passed the Energy Policy Act which was broad in scope, emphasized energy efficiency, research and development on conventional fuels, alternative fuels and uranium enrichment. The law stated that: 1) the EPA would set standards for Yucca Mountain based on findings by the National Academy of Sciences on several

specific issues related to radiological protection; 2) the NRC would provide requirements and criteria based on EPA standards, assuming engineered barriers and long-term oversight of the repository by DOE; 3) DOE would report to Congress on the adequacy of plans for disposal of waste from future reactors, and 4) states would have authority over below-regulatory-concern (BRC) wastes, negating NRC policy (Murray 164).

The principle regulation concerning low-level wastes (civilian radioactive wastes) is Part 61 (10 CFR 61) of Title 10 Energy. The legislation is based on research by the NRC and its contractors and must gain approval by industry and the public (Murray 165).

Low-level wastes are further broken down into smaller categories. "Below regulatory concern" (BRC) wastes can be disposed of without any concern of radioactivity. Next, Class A wastes require minimum precautions for disposal. They must not be stored in cardboard containers, must be solidified or mixed with an absorbent so there is no more than one percent liquid, there must be no explosive or combustible material present, containment at low pressure if gaseous, and must receive treatment if biological in origin (Murray 165). Class B wastes must be stable (keep their size and weight despite stresses on the container from soil weight, moisture and radiation) and also have to meet minimum requirements (Murray 165). Class C wastes should be protected so that an inadvertent intruder could not reach the waste by drilling, digging a well, or excavating for a building. Finally, Greater than Class C wastes (GTCC) must be treated as high-level wastes and thus are not cleared for near surface burial. The Department of Energy disposes of these wastes. These classes are based on half-lives and activity of isotopes in the material. Data for the isotopes may be found in 10 CFR 61 (Murray 165).

NRC Regulatory Guides supplement regulations and provide information on issues such as quality assurance, design bases, calculation methods and the form of reporting (Murray 165).

Over 30 pages of regulations govern the storage of high-level radioactive wastes. They are contained in Part 60 of the Code of Federal Regulations Title 10 Energy (10 CFR 60). Some of these important provisions include: 1) Design and operation of the facility should not pose an unreasonable risk to the health and safety of the public (radiation limit is a small fraction of that due to natural background); 2) a multiple barrier approach is to be used, including the waste form, containers, and host rock; 3) performance objectives are set for both the components and the system; 4) a thorough site characterization must be made, with features such as possible flooding regarded as sufficient to disqualify, and features such as geological stability or slow water flow regarded as favorable; 5) repository should be located where there are no attractive resources, far from population centers, and under federal control and should maintain good records and prominent markers; 6) high-level wastes are to be retrievable up to fifty years from the start of operation; 7) waste package must be designed to take account of all possible effects (must be dry and chemically inert); 8) wastes in the package should be safe from water for at least 300 years; and 9) predictions of safety must be made with conservative assumptions and by calculations that take account of uncertainties, using expert opinion (Murray 166).

Accompanying this legislation is 10 CFR 960, which contains the Department of Energy's criteria on characterizing repositories. In lieu of Congress's decision in 1987 to limit the study of Yucca Mountain, the regulations related to selection of several sites for

characterization and on the recommendation of one site for use are now irrelevant (Murray 166).

The fact is that nuclear regulatory policy is an area that has evolved over the last fifty years. Over that time, it has tended to be an area where fear from the people make legislation somewhat less scientific than it should be at times. As America heads into the twenty-first century and as our fuel needs continue to grow, the public must learn to look at nuclear power in a different light than it views nuclear weapons. In addition, scientists must continue to work at finding new storage techniques that are safer and cheaper than the facilities current technology permits. Nuclear power is an unlimited source of power. America should take advantage of it, but we must first learn to deal with the problems of waste disposal which come with it.

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