

## X. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

### A. SERVICE ACTIVITIES

#### 1. Breeding and Supply of Animals

During the nine months period 18195 animals were bred and 16,307 animals were supplied to various institutions including the parental organisation. However, the number of animals supplied to NIN including the animals for health monitoring is only 528 which is 2.1% of the total number of animals available. Rest of the animals were supplied to various research institutions including pharmaceutical R & D centres. The percentage of animals died or disposed were 5.95 or 4.91 respectively including mutant colonies like nude mice, Ob/Ob and GR/Ob rats. When the mutant colonies were excluded, the percentage of animals died and culled were 5 and 3.75 which is within the normal range of large colonies. The details of individual species and strains supplied are shown in Tables 29 & 30. The income generated from this activity was Rs.16.94 lakhs.

#### 2. Supply of animal feed

##### a. Stock animal feed

In the nine months period 13163 kg of rat/mice feed and 570 kg of rabbit/g.pig feed were supplied to Pharmaceutical R & D Centres and 3219 kg of rat/mice feed and 1136 kg of G.pig/Rabbit feed were supplied to Govt. institutions. The income generated from this activity was 11.97 lakhs (Table 28).

##### b. Experimental animal feed

There has been demand for making some of the experimental animal feeds as the facility has got the expertise as well as ingredients required for making experimental feed.

Table 28. During the year the following experimental animal feeds were prepared and supplied:

No.	Type of Diet	Quantity	Institution
1	A high fructose diet	14 kg	TN Medical College & Byl Nair Ch.Hospital, MUMBAI
2	Galactose diet	27.2 kg	Osmania University, Hyd.
3	Safflower oil based high fat diet	2.5kgx 8 times	Lupin Limited (Research Park) Pune
4	Tryptophan deficient diet	13 kg Exptl. and 4 kg control	Osmania University, Hyderabad
5	Iron deficient diet	20 kg	National Brain Research Centre, Nainwal More, MANESAR
6	Protein Deficient diet	20 kg	
7	High protein diet	40 kg	
8	High Fat diet	10 kg x 10 times	National Centre for Cell Sciences, (NCCS) Pune
9	High Fat diet	60%10 kg 40%10 kg 4% 10 kg	Suven Life Sciences, Hyderabad.

Apart from this, herbal powder incorporated pelleted stock diet of 320 kg was supplied to M/s. Leila Impex, Vijayawada and a hypocholesterimic oil based guinea pig feed was supplied to M/s. Shantha Biotech Ltd., Hyderabad.

### 3. Supply of Blood & Blood products

During this period a total of 445 ml of blood, sera and plasma were supplied to 8 different institutions on 32 occasions. A sum of Rs.18,740/- was realized. Apart from that 20 ml of blood was supplied to the institute.

### 4. Health Monitoring

A total of 657 samples from conventional and barrier maintained colonies were taken for microbiological monitoring during this period. This included samples from all the animal colonies maintained in the facilities. Apart from that, feed, water, bedding and equipments were also screened for microbiological screening. Organisms like *Corynebacterium* spp., *E. coli*, *Klebsiella* spp., *Proteus* spp., *Listeria monocytogenes*, *Streptococcus* spp., *Staphylococcus* spp., *Bacillus* spp., *Micrococcus* spp., and *Acinetobacter calco. var anitrat* were identified from all rat strains from both the colonies. Liver cysts were occasionally found in WNIN and SD rats from both colonies. Most of them were identified in older animals; the production and supply stock were unaffected. Once the facilities are modernized - especially increased exchange of air in the rooms - many of these organisms can be eliminated. Endoparasite, *Syphacia obveleta*, one of the common nematode was also found in most rats, especially WNIN, SD, and Fischer-344. *Giardia* spp, was frequently found in hamsters. The other common symptoms found in a wide spectrum of strains in the facility (especially in SD and WNIN rats) due to non-specific etiology were alopecia, exfoliate dermatitis, and mammary tumors. Apart from this, specific dermatitis due to ectoparasites, *Mycopites musculinus* and *Myobia* spp. were also seen in the two strains of mice BALB/c and Swiss in the facility and appropriate control measures were taken to contain them.

#### ***Sentinel Monitoring***

As explained last year, animals were identified from different age groups and placed in different areas within the colony with a specific labeling. They are being monitored for bacterial and parasitical examinations. The initial data show that most of the bacteria observed as on routine random checking (as reported above) were present in sentinels as well. Sick animals from different colonies: A total 114 animals have been reported sick during this period and they were as follows: rats 92, guinea pigs 12, rabbits 7, and Swiss mice 3., They were found to have subcutaneous tumors, hair loss, middle ear infection and severe dermatitis. These animals were physically examined and samples were collected for microbiological and histopathological examinations.

### 5. Human Resource Development

37<sup>th</sup> Annual Laboratory Animal Technicians' Course was held between 14<sup>th</sup> June and 30<sup>th</sup> July and was attended by 12 candidates.

25<sup>th</sup> Laboratory Animal Supervisor's Training Course was conducted from 1<sup>st</sup> Sept. to 30<sup>th</sup> November and 10 participants successfully completed the course.

A five day orientation programme with practical demonstration was given to 31 students of the DMLT course from Medwin Hospitals.

A total number of 11 persons were given adhoc training this year. This consists of one week orientation training in laboratory animal science (6 persons from private and government organization) as well as 3 months training in biotechnology in the molecular biology and animal physiology laboratories of NCLAS.

Table 29. Details of breeding and supply of different species and strains of laboratory animals (barrier maintained colony) during the period from 01.4.2003 to 31.03.2004.

Sl. No.	Species	Strain or Breed	Stock As on 1.4.2004	Total Number of animals						Balance as on 31.12.04	
				Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied	Died		Disp
1	Mouse	BALB/c An. N (inbred)	1170	2242	3412	216	2764	2980	1	226	205
		C57BL/6J (inbred)	631	1903	2534	-	2071	2071	104	-	359
		NIH(S) Nude (athymic) (inbred)	234	295	529	32	217	249	92	78	110
2	Rat	Wistar/NIN (inbred)	564	2424	2988	15	2163	2178	54	152	604
		SD (Sprague Dawley) (Outbred)	434	489	923	-	524	524	88	-	311
		Fischer 344 N (inbred)	49	97	146	10	32	42	23	12	69
3	G. Pig	N:HART (Hartley)	79	412	620	-	263	263	63	1	293
		Dunkin (Hartley)	135	-	-	-	-	-	-	-	-
		NIH (Coloured)	142	206	348	-	212	212	34	3	99
4	Rabbit	New zealand white	47	39	96	8	94	42	6	-	36
		TOTAL	3479	8107	11586	281	8280	8561	465	472	2088

Percentage of animals supplied to other Institutions : 71.5 %

NIN : 2.42 %

( ) Values are percentage of number of animals available in each species.

Table 30. Details of breeding and supply of different species and strains of laboratory animals (conventional colony) during the period from 1.4.2004 to 31.12.2004

Sl. No	Species	Strain or Breed	Stock As on 01.04.04	Total Number of animals							Balance as on 31.12.04	
				Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied Total	Died	Disposed		
										Old age	Sick	
1	Mouse	Swiss (Inbred)	669	3290	3909	76	2692	2768	402	70	-	669
		WNIN (Inbred)	932	5102	6034	162	4872	4932	139	218	-	985
		WNINOb-Ob (Inbred)	301	430	811	-	-	-	140	88	-	572
		WNINGR-Ob	486	425	911	9	-	9	100	206	-	586
2	Rat	Wkyto (Inbred)	133	184	317	-	12	12	40	64	-	201
		CFY/NIN (Inbred)	72	145	217	-	-	-	8	13	-	196
		Holtzman (Inbred)	222	12	234	-	-	-	100	86	-	48
		Wild White	44	3	47	-	-	-	21	-	-	26
3	Hamster	Golden (Inbred)	148	530	678	-	265	265	48	-	-	365
4	Monkey	Macaca mulatta (Rhesus)	24	-	24	-	-	-	-	-	-	24
5	Sheep		1	-	1	-	-	-	-	-	-	1
6	Rabbit		6	7	13	-	-	-	-	-	-	13
TOTAL			3108	10086	13196	247	7469	7746	1009	745	-	3696

Percentage of animals supplied to other Institutions :  
NIN :

( ) Values are percentage of number of animals available in each species.

## B. RESEARCH ACTIVITIES

### 1. PCR BASED DNA FINGERPRINTING OF WNIN STRAIN AND ITS OBESE MUTANTS

Two mutant obese rat strains, WNIN/Ob and WNIN/GR-Ob were developed from the existing WNIN rat colony, which is being maintained at NCLAS in an inbred status for the past 84 years. Both the mutants are obese, but WNIN/GR-Ob has impaired glucose tolerance additionally. The present project was undertaken to establish genetic identity for these two obese mutant rat strains. It was decided to make a DNA fingerprint profile, employing the RAPD (Randomly Amplified Polymorphic DNA) approach using random primers to establish the genetic identity. Three standard strains WNIN, WKY and Fischer-344 were used as controls. The two phenotypes of the mutants lean (+/+) and carrier (+/-) were also included for comparison.

#### Methodology

1. Genomic DNA was isolated from the blood samples of WNIN (parental Strain), WKY (related strain), Fischer-344 (unrelated strain), WNIN/Ob, WNIN/GR-Ob and their phenotypes lean (+/+) and carrier (+/-) , six replicates in each.
2. PCR was carried out using random primers (over 60) from kit A, kit B and kit E (Operon Technologies) by standard protocols. The PCR products were then run on 8% polyacrylamide gels.
3. PCR products unique to WNIN/GR-Ob were cloned into the vector PCR-2.1 TOPO and the inserts were sequenced using M13 forward and reverse primers.
4. Southern blotting and hybridization of the PCR products amplified by the primer OPB10 was carried out using <sup>32</sup>P labelled clone containing 390bp insert as probe as per standard protocol.

#### Results

1. The primer OPE7 generated initially a DNA profile unique to WNIN/Ob, but in subsequent generations, it was found to be untenable.
2. The M13 repetitive sequence, when used as a primer, also could not generate a profile unique to WNIN/Ob.
3. The (GATA)<sub>n</sub> primers and microsatellite markers linked to obesity loci also could not establish a profile unique to WNIN/Ob.
4. The random primer OPB10 generated a 'DNA fingerprint' profile for WNIN/GR-Ob which differentiated the mutant from the control strains and its two phenotypes lean and carrier and also from the other mutant WNIN/Ob.
5. The PCR products unique to WNIN/GR-Ob were of the sizes- 360bp, 390bp, 400bp and 600bp.
6. The above sequences were successfully cloned in vector pCR 2.1- TOPO and the inserts could be sequenced using M13 forward and reverse primers.
7. BLAST Search revealed that the cloned PCR products 360bp, 390bp, 400bp have homology to the sequences on rat chromosome no.3 and chromosome no.8. The 600 bp PCR product has partial homology to chromosome X (only upto 150 bp).
8. Southern blotting and hybridization of the PCR products with the clone containing 390bp insert as probe showed hybridization to the WNIN/GR-Ob indicating that the cloned regions are part of the rat genome.

## Conclusions

The efforts to generate a DNA fingerprint unique to WNIN/Ob were not successful. However, a 'molecular signature' specific to the WNIN/GR-Ob mutant could be generated and the unique PCR products thus generated have homology with chromosome no.3 and chromosome no.8 and partially to chromosome X.

## 2. GENETIC TYPING OF WNIN/Ob AND WNIN/GR-Ob STRAINS USING MICRO-SATELLITE MARKERS

The two obese mutant rats developed from WNIN rat colony at NCLAS, WNIN/Ob and WNIN/GR-Ob need to be genetically typed to establish a DNA profile unique to them. Both the mutants are obese, but WNIN/GR-Ob has impaired glucose tolerance additionally. Microsatellite markers are abundant, randomly distributed throughout the genome, polyallelic and are genetically more informative. Therefore, approximately 100 microsatellite markers were selected, spanning all the 20 chromosomes and the X chromosome taking three to four markers per chromosome to type the obese mutant strains. Three standard strains, WNIN, WKY and Fischer-344 were used as the controls and the two phenotypes of the mutants, lean (+/+) and carrier (+/-) were also included in the study.

### Methodology

1. Genomic DNA was isolated from the blood samples of WNIN (parental strain), WKY (related strain), Fischer-344 (unrelated strain), WNIN/Ob and WNIN/GR-Ob and their phenotypes lean and carrier, six replicate in each group.
2. PCR was carried out using microsatellite markers by the method by Serikawa et al (1992). The PCR products were then run on 12% polyacrylamide gels.
3. Hierarchical cluster analysis using centroid method was performed on the data generated using SPSS package version 11.5.

### Results

1. A genomic scan of the standards and the mutant strains was performed using 96 microsatellite makers and out of which, 62 primers yielded good genetic profiles.
2. Out of these 62 primers, 9 microsatellite markers were found to be useful for rat strain identification.
3. Cluster analysis of 62 microsatellite primers based data, indicated the formation of two clusters; the first one contained the parental strain WNIN and the mutants, WNIN/Ob and WNIN/GR-Ob, the second cluster contained the standard strains -WKY and Fischer-344.

## Conclusions

Nine primers were identified which can be used for genetic monitoring of rat strains. Amongst the nine, the microsatellite, primer leukosianin has great promise as it could detect length polymorphism between the three standard rat strains and also differentiate the mutant obese strain from the parental strain WNIN.