CHAPTER - II

MATERIALS AND METHODS

MATERIALS AND METHODS

The present studies were concerned with effects of manipulating the plane of nutrition with regard to selected nutrients at different ages in the rat. The parameters measured varied with the nutrient manipulated and the age during treatment. The following experiments were conducted :

(1) Growth rate, feed utilization, behavioral responses in the open field and activity wheel, reproductive performance and maternal behavior in rats subjected to different degrees of pre-weaning and/or postweaning food restriction.

(2) Comparative studies on two generations of rats fed diets simulating those consumed by different population groups with regard to reproductive performance, nutritional status and nitrogen balance during gestation and lactation.

(3) Response of rats to variations in dietary protein content and to changes in the same as judged by growth, nutritional status, nitrogen balance and incorporation of a labelled amino acid in serum protein.

(4) Response of rats fed on a wheat diet with and without addition of lysine at different ages as judged by growth, nutritional status and incorporation of a labelled amino acid in serum protein.

(5) Response of bone composition to different amountsof dietary calcium in relationto age at treatment.

(6) The utilization of carotene in rats depleted of vitamin A in relation to dietary vitamin A source (vitamin A or carotene) prior to depletion.

Albino rats of the Charles Foster strain, bred in the departmental stock colony were used for these studies. For the studies after weaning age, the animals were weaned at 21 days of age and those assigned to the different groups were matched for age, sex and body weight, litter-mates being used in most cases.

Design of the experiments

Growth and food utilization of rats subjected to different degrees of pre-weaning and/or postweaning food restriction. Studies were carried out to investigate the effects of food restriction in the neonatal and/or postweaning periods on the growth and efficiency of food utilization of male and female rats. For this purpose, post parturient females which delivered on the same day were assigned 4, 8 or 12 pups per litter in order to manipulate the plane of nutrition during the neonatal period. At weaning the animals were divided into three groups according to weaning weights i.e. 45-50 g, 35-45 g and 25-35 g. Each group was further divided into four subgroups. One was fed a qualitatively adequate diet ad libitum. For the other three groups the amounts of food given were adjusted to 80, 66 and 50% of the amount consumed by the first group. After a period of 8 weeks,

all the animals were switched over to an ad libitum feeding schedule till the termination of the experiment. Body weights were recorded once a week. Food intake of the controls was recorded for two days in a week. Food intake was recorded on a dry weight basis as the difference between the amount given and the amount left. Food spilled was collected carefully to determine the latter. Efficiency of food utilization was calculated as amount of tissue gained per gram of dry food. Studies were made of variations in the same in relation to the plane of nutrition, age and sex.

Experiment 1(b)

Behavioral responses in the open field and activity wheel of male rats subjected to different degrees of pre-weaning and/or postweaning food restriction.

Studies were made of the effects of early food restriction induced during the neonatal and/or immediate postweaning period on subsequent activity of male rats. The males used in Experiment 1(a) were tested for their performance in an open field for 6 consecutive days at the age of 16 weeks, i.e. 5 weeks after the switch to an ad lib feeding schedule. At the end of this period, the animals were rested for a day and tested for performance in an activity wheel. The procedures will be described elsewhere.

Experiment 1(c)

Reproductive performance of female rats subjected to different degrees of preweaning and/or postweaning food restriction.

For the studies on the effects of early food restriction on subsequent reproductive performance and maternal behavior, the females in Experiment 1(a) were kept for mating when they were 16 weeks of age, i.e., 5 weeks after the switch to an ad lib feeding schedule for all the groups. Well fed healthy males from the stock colony were used as mates, Reproductive performance was studied with respect to maternal food intake and weight change during gestation and lactation, the number of pups born, the number surviving at weaning, and growth rate of pups.

Experiment 1(d)

Maternal behavior of rats subjected to different degrees of preweaning and/ or postweaning food restriction.

Maternal behavior was studied in the above animals on the 5th and 6th day of lactation according to the method described by Frankova (1974). The stock diet was used for this experiment. The composition of the diet is given in Table 7.

Table 7 : Composition of the stock diet. (Experiment 1).

Ingredient	Amount (g) per kg diet
Wheat flour (Triticum sativum)	350
Bajra flour (Bennicitum glaucum)	100
Bengal gram flour (Cicer arietenum)	110
Milk powder*	210
Sprouted legumes**	160
Groundnut oil (Peanut oil)	70
Vitamin A***	5000 i.u.

* Floor sweepings which contained skim and whole milk powder were obtained from Amul Dairy, Anand.

** A mixture of 80 g. cow peas (Vigna catjung) and 80 g. green gram (Phaseolus mungo) was used.

*** 70 g fenugreek leaves (Brassica nigra) were used when in season. Otherwise, vitamin A acetate in oil was added.

Experiment 2

Comparative studies on two generations of rats fed diets consumed by different population groups with regard to reproductive performance, nutritional status, and nitrogen balance during gestation and lactation.

The studies on the effects of varying the overall quality of diet on reproductive performance were made through two generations. For this purpose, 8-12 week old females were fed diets typical of the diets consumed by the low income group (LIG) and high income group (HIG) in Gujarat. A third group was fed a typical Western diet (W). The reproductive performance of the rats in the three groups in terms of the criteria specified above was studied. The animals were allowed to mate at 12-18 weeks of age and continued on the respective diets through succeeding generations. Determinations were made of blood hemoglobin and serum protein before gestation (i.e. just before mating) on day 13-15 of gestation and on day 12 of lactation. For mating, females were kept with their prospective mates for only 3 days and if pregnancy resulted, the day of conception was taken as day 2 of this period. This wouldresult in an error of not more than one day in the estimation of gestational age. This procedure has been found convenient in several previous studies in this laboratory. In this connection, it may be mentioned that Stewart (1973) has used a similar procedure to ascertain gestation time. Nitrogen

balance studies were also conducted on these animals prior to mating, for three consecutive days and between days 14-17 of gestation. Nitrogen balance studies were also carried out during 12-18 days of lactation according to the procedure described later.

The female progeny of these animals were continued on the respective diets for 12-14 weeks after weaning. They were then mated with well-fed males from the stock colony and data on reproductive performance and biochemical parameters were collected as for the previous generation.

Diets typical of the LIG and HIG groups were formulated on the basis of diet surveys made in this laboratory (Rajalakshmi, 1975) and of the Western diet according to data given by Greaves and Hollingsworth (1966) of U.K. (Table 8). An average monthly consumption pattern of vegetables and fruits was formulated on the basis of surveys carried out in Baroda (Subbulakshmi, 1970; Rajalakshmi et al, 1978) (Table 9). The vegetables were purchased in bulk, washed, cut, immersed in boiling water using a colander for 5 minutes, dried in a hot air oven at 60-80°C for 36-48 hrs. and powdered. The dry powder was stored in plastic containers at 10°.

A problem was faced in the administration of vegetables and fruits. The idea was to simulate the diets consumed, but the vegetable mixture used was based on annual consumption

Table 8 : Food consumption pattern in low and high income groups in Baroda as compared to values reported for the British[1] (Experiment 2).

Ingredient (g)	LIG	HIG	Western
Miled flour of :			
Wheat (Triticum sativum)	100	150	220
Rice (Oryza sativum)	50	100	-
Bajra (Pennicitum glaucum)	150	-	-
Kodri (Paspalum scorbiculatum)	50	-	-
Moth bean (Phaseolus aconitifolius)	3	-	-
Bengal gram dal (Cicer arietenum)	12	10	-
Red gram dal (Cajunus cajan)	12	30	-
Green gram dal (Phaseolus mungo)	3	10	-
Milk powder[2]	12	65	65
Sugar	15	30	138
Jaggery (Brown sugar)	10	15	-
Fat			
Groundnut oil	15	30	-
Hydrogenated oil (Dalda)	-	15	44

contd...

Table 8 : contd.

Ingredient (g)	LIG	HIG	Western
Butter (Amul)	-	-	25
Meat[3]	10	-	250
Leafy vegetables[4]	18	36	75
Other vegetables			
Fruit			
Common salt	10	10	10
Cheese (Cheddar)	-	-	12
Peanuts	-	15	35

1. The values for the three groups were taken from Rajalakshmi and Ramakrishnan, (1977). The data used in this source are based on previous studies in this laboratory for the first two groups and on the report of Greaves and Hollingsworth (1966) for the British.

2. 12 g milk powder taken as 100 ml milk. Floor sweepings of milk powder were obtained from Amul Dairy, Anand.

3. Minced mutton was autoclaved and stored in polythene bags at 0°. Since 80% of the families in the High Income Group are vegetarians, no meat was added to the diet of this group.

4. The monthly consumption pattern of vegetables and fruit was formulated in the basis of some surveys carried out in Baroda (Rajalakshmi and Ramakrishnan, 1969(b); Subbulakshmi, 1970; Rajalakshmi et al,1978) and is given in Table 6. Vegetables and fruit were purchased in the proportions given in this table and processed as described in the text. Hundred g of fresh vegetables gave on an average 15 g of dry powder.

Table 9 : Pattern of vegetable consumption in different groups (Experiment 2).

Vegetables and fruits (g)	LIG[1]	HIG[1]	Western[2]
Potato (Solano tuberosum)	770	1400	7500
Brinjal (Solanum melongena)	360	400	-
Bottle gourd (Lagneria siceraria)	150	150	-
Onion (Allium capa)	250	250	450
Tomato (Lycopersicon esculentum)	40	200	1500
Beans (Phaseolus vulgaris)	180	200	300
Cauliflower (Brassica oleracea, variety botrytis)	80	200	300
Tindola (Coccinacordifolia)	75	200	-
Elephant yam (Dioscorea species)	70	100	-
Radish (Raphanus sativus)	60	60	-
Bitter gourd (Mimordica charantia)	50	200	-
Cabbage (Brassica oleracea variety : capitata)	50	200	450
Pumpkin (Cucurbita pepo)	-	50	-
Ladies finger (Hibiscus esculentus)	75	200	-

contd...

Table 9 : contd.

Vegetables and fruits (g)	LIG[1]	HIG[1]	Western[2]
Parwal (Trichosanthes dioica)	80	100	-
Kankoda (Mimordica dioica)	-	50	-
Drumstick (Moringa oleifera)	25	50	-
Peas (shelled)	-	100	450
(Tuar (shelled) (Cajunus cajan)	50	100	-
Carrot (Daucus carota variety : sativa)	-	100	450
Lettuce (Lactuca sativa)	-	-	45
Fenugreek leaves (Brassica nigra)	240	400	-
Banana (Musa paradisiaca)	80	350	900
Apple (Malus sylvestris)	-	50	1200
Orange (Citrus sinensis)	-	50	1200
Guava (Psidium guajava)	10	25	-
Chikoo	10	25	-

1. Based on Rajalakshmi and Ramakrishnan (1969(b)); Subbulakshmi (1970); Rajalakshmi et al,(1978).

2. The values were obtained by conducting dietary surveys eliciting daily information on the fruit and vegetable consumption of the whole family in the case of LIG and HIG groups in Baroda and on the basis of food supplies in the United Kingdom as given by Greaves and Hollingworth (1966) for the Western group.

rather than day to day intakes. This necessitated the purchase of same of the non-seasonal vegetables in bulk. Problems were also faced in the administration of the vegetables and fruits as the animals tended to gat them up first and it was feared that this might interfere with the intended composition of the diet if the other foods were not consumed in the expected proportions. This also made the estimations of food intake difficult. To overcome these problems the vegetables were dried at 60-80° powdered and stored in the refrigerator for a few weeks and added to the diet at the time of consumption. It is realised that some vitamin losses would have taken place with, this procedure, but these would be no more than the losses incurred during cooking in this region. The diets fed were found to be reasonably adequate with regard to the B vitamins even without taking into account the contributions of the vegetables. The marginal deficiencies of riboflavin and vitamin A are no more than are found in the diets actually consumed (Table 10).

Experiment 3

Response of rats to variations in dietary protein content and to changes in the same as judged by growth, nutritional status, nitrogen balance and incorporation of a labelled amino acid into serum protein.

Studies were made of the effects of feeding diets containing different levels of dietary protein on immediate and subsequent growth, blood hemoglobin, serum protein, nitrogen

Table 10 : Vitamin content* of diets consumed by different groups (Experiment 2).

Diet		B_1	B_2	Niacin	Vit.A/ Carotene µg/day
		mg per 1000 Kcals			
	Requirement	0.4	0.55	5.00	750
LIG	Present in diet excl. veg.	0.80	0.34	6.16	25
	Present in raw veg. mix.	0.04	0.035	0.43	237
	Present in diet incl. veg. mix. after allowing 50% loss in veg. mix.	0.82	0.36	0.37	156
HIG	Present in diet excl. veg.	0.56	0.48	5.90	124
	Present raw veg. mix.	0.06	0.05	0.67	704
	Present in diet incl. veg. mix.	0.59	0.51	6.23	476
Western	Present in diet excl. veg.	0.44	0.39	7.58	194
	Present in raw veg. mix.	0.11	0.06	1.46	1488
	Present indiet incl. veg. mix.	0.51	0.41	8.31	938

* Based on food composition tables for use in East Asia FAO (1972).

balance and incorporation of U-C^{14} -DL-Leucine in serum protein.

Weanling rats were fed ad libitum diets containing 5,10 or 20% protein in the form of casein. After 8 weeks of treatment, half of the 5% and half of the 20% animals were switched over to a 10% diet. After afurther period of 12 weeks, two of the 5%-10% animals were switched back to a 5% diet (5%⟶10%⟶5% group). Two of the 10% animals were switched to a 5% diet (10% ⟶ 5%) 21 weeks after starting of the experiment. The various switches of diet were made in view of the observations made during the course of the experiment and are discussed in appropriate context. Body weights were recorded once a week throughout the experiment. All other estimations were made 7 and 15 weeks after the start of the experiment.

The composition of the diets used is given in Table 11.

Experiment 4

Response of rats fed on a wheat diet with and without addition of lysine at different ages as judged by growth, nutritional status and incorporation of a labelled amino acid in serum protein.

(a) Studies were made of the effects of lysine supplementation to a wheat diet at different ages on growth, blood hemoglobin, serum protein, liver protein and incorporation of U-C^{14} -DL-leucine into serum protein.

Table 11 : Composition of diets containing different levels of protein (Experiment 3).

	Per cent protein in diet		
	5	10	20
	amount (g) per 100 g.		
Casein	6	12	24
Sago	63	63	63
Salt mixture*	4	4	4
Vitamin mixture**	2	2	2
Sugar	18	12	-
Groundnut oil	7	7	7

** Composition given in Table 17.

* Composition given in Table 18.

Groups of male rats aged 3, 13, 26 or 52 weeks were fed either a wheat diet or the same supplemented with lysine for 8 weeks. At the end of the experiment, tail blood samples were analysed for blood hemoglobin, serum protein and incorporation of U-C^{14} -DL-leucine into serum protein. The animals were decapitated at the end of treatment, their livers removed and estimated for protein content.

(b) As an extension of the previous experiment, studies were made of the effects of prolonged feeding of a wheat or wheat + lysine diet to weanling rats.

Weanling male rats were fed either of the diets for a period of 22 weeks. At the end of this period, data on blood hemoglobin, serum protein and incorporation of U-C^{14} -DL-leucine into serum protein were obtained. Half of the animals from each group were then switched over to the diet of the other group i.e. from wheat to wheat + lysine and vice versa and the experiment terminated after a further period of 10 weeks.

The composition of the diets used is given in Table 12.

Experiment 5

Response of bone composition to different amounts of dietary calcium in relation to age at treatment.

Studies were made of theeffects of variations in dietary calcium levels on growth, food intake, Blood hemoglobin, serum

Table 12 : Composition of wheat and wheat + lysine diets (Experiment 4).

Ingredient (g) per 100 g. diet	Diet	
	Wheat	Wheat + lysine
Wheat (Triticum sativum)	87	87
Salt mixture*	4	4
Vitamin mixture**	2	2
Groundnut oil (Peanut oil)	7	7
L-Lysine	-	0.2

** Composition given in Table 17.

† Composition given in Table 18.

protein and bone composition at different ages. The reversibility of the effects, if any, to low and high calcium diets was investigated by switching the animals to a standard diet.

Groups of males aged, 3, 13, 26 or 52 weeks were fed for 5 weeks, diets providing, per 100 g., 100, 440 or 600 mg calcium. Half the animals from each group were killed at the end of this period. Data were obtained on blood hemoglobin and serum protein just before slaughter, and bone composition, shortly after. The remaining animals were switched over to a diet containing a standard amount of calcium (440 mg per 100 g) and the experiment continued for another 4 weeks. Data were obtained on the parameters specified above.

The composition of the diets and salt mixtures used for this experiment is given in Tables 13 and 14.

Experiment 6

The utilization of carotene in rats depleted of vitamin A in relation to dietary vitamin A source (Vitamin A or carotene) prior to depletion.

For the studies on the effects of feeding carotene or vitamin A in early life on subsequent utilization of carotene, weanling male rats were fed diets based on kodri (Paspalum scorbiculatum) + cowpeas (vigna catjung). Neither ingredients contains a measurable amount of carotene. Known amounts of carotene or vitamin A were added for a period of 5 weeks after

Table 13 : Composition of diets providing different levels of calcium (Experiment 5).

Ingredient (g)	Diet providing per 100 g.		
	100 mg Ca	440 mg Ca	600 mg Ca
Casein	24.0	24.0	24.0
Sago	64.7	63.0	62.2
Salt mixture*	2.3 (I)	4.0 (II)	4.8 (III)
Vitamin mixture**	2.0	2.0	2.0
Groundnut oil (Peanut oil)	7.0	7.0	7.0

* These variations were made so as to manipulate calcium content without affecting the amounts of other minerals provided. See Table 14.

** Composition given in Table 17.

Table 14 : Composition of salt mixtures providing different amounts of calcium (Experiment 5).

	Salt mixture		
	I	II	III
Calcium (mg per 100 g diet)	100	440	600
Amount added to 100 g. diet	2.3	4.0	4.0
Calcium citrate	-	308.2	308.2
$Ca(H_2PO_4)_2H_2O$	-	112.8	112.8
K_2HPO_4	208.7	208.7	324.5
KCl	124.7	124.7	124.7
NaCl	77.0	77.0	77.0
$CaCO_3$	60.5	68.5	168.5
$MgSO_4$	38.3	38.3	38.3
$MgCO_3$	35.1	35.1	35.1
Salt mixture 'A'**	16.7	16.7	16.7
Ca : P ratio	0.65	2.3	2.3

** Salt mixture 'A' contained, per 100 g. ferrous ammonium citrate, 91.41 g; copper sulfate, 5.98 g; sodium flouride, 0.76 g; magnesium sulfate, 1.07 g; potassium aluminium sulfate, 0.54 g; and potassium iodide, 0.24g.

which the animals were fed a vitamin A/carotene free diet and their body weights monitered weekly. The diets were continued till the animals showed evidence of growth retardation followed by virtual growth arrest. This phase lasted 13 weeks. Serum vitamin A level was estimated at this stage from tail blood samples. The rats were then fed a diet containing 150 µg carotene for 3 weeks after which serum and liver vitamin A were estimated.

The composition of the diet used for this experiment is given in Table 15. Fenugreek leaves were purchased in bulk, washed, dried in a hot air oven at 60-80°C for 18-24 hrs and powdered. The dry powder was assayed for carotene. It was stored in a plastic container at about 10°C. The various experiments described above are summarized in Table 16.

Procedural details

Water was provided ad libitum. So was food except where food restriction was part of the experimental procedure. Body weights were recorded once a week. Food intake was determined twice a week in the case of animals fed ad lib. As the food-restricted animals ate up all the food provided, this was not necessary in their case.

The animals were housed individually in small galvanised iron cages (8" x 6" x 8") except for studies on reproductive performance. During mating, two females were kept with one male

Table 15 : Composition of diet providing vitamin A or carotene (Experiment 6).

	Amount (g) per 100 g. diet	
	Vitamin A	Carotene
Kodri (Paspalum scorbiculatum)	80	80
Cow peas (Vigna catjung)	20	20
Salt mixture*	4	4
Vitamin mixture**	2	2
Groundnut oil (Peanut oil)	7***	7
Dried fenugreek leaves	-	3@

* For composition, see Table 18.

** For composition, see Table 17.

*** 50 µg vitamin A acetate is added to the oil.

@ Dried sample estimated to contain about 150 µg carotene. About 33% of the carotene in fresh leaves was destroyed due to drying.

Parameters measured	Additional details
(a) Body weight, Food intake	(a) done on all animals
(b) Open field activity, activity in an activity wheel	(b) only males tested at 16 weeks of age.
(c) Maternal weight change, food intake, survival and growth of pups from birth to weaning.	(c) females kept for mating at 16 weeks of age.
(d) Maternal behavior	(d) measured on days 6 and 7 of lactation.
Maternal weight change and food intake during gestation and lactation, litter size, survival and growth of pups from birth weaning, blood hemoglobin, serum protein and nitrogen balance during gestation and lactation.	The animals were raised on the stock diet prior to their inclusion in the experiment and fed the test diets for 4-6 weeks before mating, Groups GI, GII and GIII consisted of representative samples of the pups derived, chosen by selecting 1-2 pups from each litter.
Body weights, blood hemoglobin, serum, nitrogen balance, incorporation of U-C-DL-leucine into serum protein.	Data for males and females were recorded and analysed separately.

Parameters measured	Additional details
Body weights, blood hemoglobin serum protein liver protein & incorpDration of U-C^{14}-DL-leucine into serum protein.	Dm Groups II, III and IV animals had beeh raised on the stock diet fed ad lib prior to their induction in the experiment.
Body weights, blood hemoglobin, serum protein, incorppration of U-C^{14}DL-leucine into serum protein.	
Body weights, food intake, blood hemoglobin, serum protein, bone composition.	
Body weight, serum vitamin A, liver vitamin A.	Animals were depleted of vitamin A in Phase II. Cessation of growth was taken as the index of depletion.

in breeding cages (12" x 12" x 12") for 3 days in Experiment 2 and 4-6 days in other experiments. Thereafter the females were housed individually in the breeding cages throughout gestation and with their pups during the suckling period. Thin strips of paper were provided as nesting material around 10-15 days of gestation according to established practice in this laboratory.

Preparation of diet

Edible casein used for experimental diets was obtained from Amul Dairy, Anand. It was washed first with 95% ethanol and then washed free of alcohol with tap water. The washed casein was dried at 27-30° and sstored in tins. Nitrogen content wasestimated by the microkjeldahl method (Hawk, Oser and Summerson, 1954) and protein calculated as N X 6.25 g per 100 g casein. Commercially available sago prepared from tapioca flour (Manihot utilissima) was ground and used as starch source as it contains only 0.2 per cent protein and no more than traces of vitamins and minerals. As tapioca flour is processed to some extent during the preparation of sago the starch in the same is believed to be readily available (Bocher, Behan and McMeans, 1951). Vitamins and minerals were provided in adequate amounts except where manipulation of the samewas part of the experimental design as in the studies on calcium. The vitamin mixture used was formulated previously in this laboratory on the basis of the allowances suggested by Brown

and Sturtevant (1949), recommendations made by the NAS-NRC (1962) and evidence reviewed by Mitchell (1964). The salt mixture used was the Hawk, Oser salt mixture No. 3 (Hawk, Oser and Summerson, 1954) unless stated otherwise. Composition of the vitamin and mineral mixtures are given in Tables 17 and 18 respectively.

For preparing natural diets, the millets, cereals, pulses, sago and groundnut oil (peanut oil) were purchased in bulk from the local market. Readily perishable commodities such as butter, cheese, meat and hydrogenated fat were purchased in smaller quantities and stored at about 10^{o}. Meat was stored at 0^{o}. Casein and whole milk powder were purchased from Amul Dairy, Anand.

Chemicals

All the chemicals used were of research grade purity. Since the quality of the chemicals procured sometimes changes from lot to lot, they were procured in bulk so as to last for the entire experiment and used. The sources from which they were obtained are indicated in Table 19.

Collection of blood

Blood samples were obtained from the tail vein. The tail was rubbed 2-3 times with xylene to stimulate blood circulation. The tip (2-3 mm) of the tail was then cut with a pair of sharp

Table 17 : Composition of vitamin mixture.

Vitamin	Amount per kg diet
Thiamine (mg)	1.5
Riboflavin (mg)	2.5
Pyridoxine hydrochloride (mg)	1.0
Niacin (mg)	15.0
Calcium-d-pentothenate (mg)	10.0
Choline chloride (mg)	750.0
Inositol (mg)	200.0
p-amino benzoic acid (mg)	10.0
Folic acid (mg)	1.0
Cyanocobalamine (µg)	5.0
Biotin (µg)	1.0

Powdered sugar was added to make a total weight of 20 g.

Table 18 : Composition of salt mixture.

	Amount (g) per kg diet
Calcium citrate	308.2
Calcium dihydrogen phosphate	112.8
di-potassium hydrogen phosphate	218.7
Potassium chloride	124.7
Sodium chloride	77.0
Calcium carbonate	68.5
Magnesium sulfate (anhydrous)	38.3
Magnesium carbonate (light) $(3MgCO_3.Mg(OH)_2.3H_2O$	35.1
Salt mixture 'A'*	16.7

* Salt mixture 'A' contained (g) per100 g; Ferrous Ammonium citrate, 91.41; copper sulfate 5.98; sodium flouride, 0.76; Manganese sulfate, 1.07; potassium aluminium sulfate,0.54; potassium iodide, 0.24.

Table 19 : The sources of the chemicals used.

Sr. No.	Chemical	Source
1.	Alcohol (absolute)	Alembic Chemical Works, Baroda
2.	Ammonia solution (25%) (G.R.)	Sarabhai M. Chemicals, Baroda
3.	Ammonia molybdate	Pfizer (India) Ltd., Bombay
4.	1-Amino-2-napthol-4-sulfonic acid (LR)	BDH (India) Pvt. Ltd., Bombay
5.	d-Biotin	Haffmann-LaRoche Inc.
6.	Boric acid (LR)	Sarabhai M. Chemicals, Baroda
7.	Bovine serum albumin	Sigma Chemical Co., U.S.A.
8.	Bromo cresol green (A.R.)	Koch Light Labs. Ltd.
9.	Calcium carbonate	Sarabhai M. Chemicals, Baroda
10.	Calcium citrate	Chemical Centre, Baroda
11.	Calcium dihydrogen phosphate (LR)	Sarabhai M. Chemicals, Baroda
12.	Calcium-d-pantothenate (LR)	BDH (India) Pvt.Ltd., Bombay
13.	Chloroform	Sarabhai M. Chemicals, Baroda
14.	Choline chloride	Sarabhai M. Chemicals, Baroda
15.	Copper sulfate	BDH (India) Pvt.Ltd., Bombay
16.	Cyanocobalamine I.P.	Merck Sharpe & Dohme of India Ltd.
17.	Ferrous ammonium citrate	Sarabhai M. Chemicals, Baroda
18.	Ferric chloride	Sarabhai M. Chemicals, Baroda
19.	Folic acid	Sigma Chemical Co., U.S.A.

contd...

Table 19 : contd.

Sr. No.	Chemical	Source
20.	Hydrochloric acid (conc)	Shreyas Chemicals, Baroda
21.	meso-Inositol (A.R.)	E. Merck, Germany
22.	DL-Leucine-1-C^{14}	Bhabha Atomic Research Centre Bombay
23.	Lithium sulfate	Sarabhai M. Chemicals, Baroda
24.	L-Lysine mono hydro-chloride	BDH Chemicals Ltd., England
25.	Magnesium carbonate (light)	Sarabhai M. Chemicals, Baroda
26.	Magnesium sulfate (anhydrous)	Sarabhai M. Chemicals, Baroda
27.	Manganese sulfate	Sarabhai M. Chemicals, Baroda
28.	Methyl red	Reanal, Budapest, Hungary
29.	Methanol	Sarabhai M. Chemicals, Baroda
30.	Niacin	Loba-Chemie-Wien Fischamend, Austria
31.	Oxalic acid (LR)	Sarabhai M. Chemicals, Baroda
32.	Para-amino benzoic acid	BDH (India) Pvt. Ltd.
33.	Phosphoric acid (ortho) (85%)	Pfizer India Ltd., Bombay
34.	Potassium chloride	Sarabhai Chemicals, Baroda
35.	di-potassium hydrogen phosphate	Sarabhai M. Chemicals, Baroda
36.	Potassium aluminium sulphate	Sarabhai M. Chemicals, Baroda
37.	Potassium iodide	Sarabhai M. Chemicals, Baroda
38.	mono-potassium phosphate	Sarabhai M. Chemicals, Baroda

contd...

Table 19 : contd.

Sr. No.	Chemical	Source
39.	Potassium permanganate	Pfizer (India) Ltd., Bombay
40.	Potassium sulfate	Sarabhai M. Chemicals, Baroda
41.	PPO (2,5,diphenyl oxazole)	V.P.Chest Institute, Delhi
42.	POPOP (1,4,bie-5-phenyl oxazole-2-yl-benzene)	V.P. Chest Institute, Delhi
43.	Pyridoxine hydrochloride (LR)	BDH (India) Pvt. Ltd., Bombay
44.	Riboflavin	BDH Chemical Ltd., England
45.	Sodium acetate	BDH (India) Pvt. Ltd., Bombay
46.	Sodium bisulfite	Sarabhai M. Chemicals, Baroda
47.	Sodium carbonate	Sarabhai M. Chemicals, Baroda
48.	Sodium chloride	Sarabhai M. Chemicals, Baroda
49.	Sodium flouride	Sarabhai M. Chemicals, Baroda
50.	Sodium hydroxide	Sarabhai M. Chemicals, Baroda
51.	Sodium molybdate	Pfizer (India) Ltd., Bombay
52.	Sodium potassium tartarate	Sarabhai M. Chemicals, Baroda
53.	Sodium sulfate	Sarabhai M. Chemicals, Baroda
54.	Sodium sulfite (anhydrous)	Sarabhai M. Chemicals, Baroda
55.	Sodium tungstate	Pfizer (India) Ltd., Bombay
56.	Solvent ether	Alembic Chemical Works, Baroda
57.	Sulfuric acid (conc.) (A.R.)	Shreyas Chemicals, Baroda

contd...

Table 19 : contd.

Sr. No.	Chemicals	Source
58.	Thiamin hydrochloride	Loba-Chemie Wien Fischamend, Austria
59.	Trichloroacetic acid	Riedel-de Haen, Hanover.
60.	Trifluoroacetic acid	BDH (India) Pvt. Ltd., Bombay
61.	Toluene (G.R.)	Reechem Pvt. Ltd., Hyderabad
62.	Vitamin A acetate	E. Merck
63.	Xylene	Pfizer (India) Ltd., Bombay

scissors and blood collected in a micropipette for hemoglobin estimation and in glass capillaries (diameter 1 mm) for obtaining serum. The tail tip was dabbed with alcohol to prevent infection. The glass capillaries were sealed with semi molten paraffin wax. They were allowed to stand at 27° for at least one hour for separation of serum. They were then centrifuged in a clinical centrifuge at low speed (1000 rpm) for 2-5 minutes. The capillary was cut to remove the blood clots and resealed. The serum thus obtained was stored at 10°.

Separation of the tissues

Where tissue analysis was part of the experiment, the animals were decapitated after starving them overnight and the liver and femur bones removed immediately, were freed of all extraneous tissue, weighed and used for analysis.

Biochemical assays

Blood hemoglobin

The method described by Varley (1969) was used. Twenty ul of blood were mixed with 8 ml of 1% ammonia solution and the colour read at 540 mu against a blank containing 1% ammonia solution in a Klett-Summerson Colorimeter.

Serum protein

The method described by Varley (1969) was used. Twenty µl of serum were taken in a serological tube (0.8-1.0 mm x 5 cm)

and 0.3 ml of sulfate-sulfite reagent and 0.8 ml of working biuret reagent added. The tube was shaken well and incubated at 37° for 20 min. The colour developed was read at 540 mμ in a Beckman Spectrophotometer, using a micro attachment, against a blank containing 0.3 ml sulfate-sulfite reagent and 0.8 ml working biuret reagent. A standard graph was obtained using different concentrations of standard serum (ref. Table 20) and serum protein was calculated therefrom.

Incorporation of U-C^{14} -DL-leucine into serum protein

The method used by Rao and Radhakrishnan (1966) for studying the incorporation of a labelled amino acid into various rat tissues was used after suitable modifications. Animals were starved for 24 h before injecting labelled leucine. Five μCi per 100 g body weight of DL-leucine-U-C^{14} (Sp. act. 12.3) was injected intraperitoneally. Tail blood collections were made at specific time intervals after injection and serum obtained.

Protein from 0.02 ml of serum was precipitated by adding 1 ml of 10% trichloroacetic acid (TCA). The tube was centrifuged in a clinical centrifuge at 3000 rpm for 10 minutes, the supernatant discarded and the residue processed further according to the method described by Hall and Cocking (1965). The residue was washed successively with 5 ml each of 95% ethanol, ethanol-solvent ether (1:1) mixture and solvent ether to remove TCA and lipids. After the final ether wash, the residue was dissolved in 1 ml of 1N sodium hydroxide. 0.2 ml

of this was taken in a counting vial. Counting was done by the method described by Perlman and associates (1964) for aqueous solutions. 4.2 ml of scintillation liquid and 3 ml of absolute alcohol were added to the vial. A white blob was formed which disappeared on swirling. A drop of glacial acetic acid was added to neutralise the alkali and get a clear solution. Counting was done in a liquid scintillation counter (Electronic Corporation of India Ltd., LSS 34) having a counting efficiency of 80%.

Specific activity was calculated as counts per minute (cpm) per g serum protein.

Liver protein

The method described by Lowry et al (1951) was used. A 10% homogenate of liver was prepared by homogenising the tissue in a Potter-Elvehjam homogeniser for 1 min using 0.9% sodium chloride as grinding medium. 0.2 ml of the homogenate was taken in a test tube to which was added 1 ml of 10% trichloroacetic acid, and the mixture centrifuged in a clinical centrifuge at 3000 rpm for 10 min. The supernatant was discarded and the precipitate was dissolved in 10 ml of 0.1N sodium hydroxide. 0.5 ml of this solution was taken and diluted to 1 ml with 0.1N sodium hydroxide and treated with 5 ml of Lowry's C and 0.5 ml Folin's reagents. The tube was allowed to stand for 30 minutes at 27^{o} and the colour developed read at 660 mμ in a Klett-Summerson Colorimeter against a blank containing

1 ml 0.1N sodium hydroxide, 5 ml Lowry's C and 0.5 ml Folin's reagent. A standard graph was obtained using aliquots varying from 20 to 100 µg of bovine serum albumin and liver protein calculated therefrom.

The composition of bone (femur)

Moisture : The femurs were freed of adhering tissue and dried in a hot air oven regulated to maintain 60° till constant weights were obtained. The loss of weight was taken as moisture content.

Fat free dry weight : The dry bone was immersed in petroleum ether (40°-60° grade) in a test tube at room temperature (27° - 30°) for 24 h. The fat free femur was dried at 60° weighed and used for the estimation of ash, calcium and phosphorus.

Ash : The fat free dry femur was taken in a previously ignited, cooled and weighed crucible, charred and then incinerated at 550-600° in a muffle furnace till it was completely ashed and the weight of the ash determined.

Calcium : The method described by the A.O.A.C. (1950) was used. Bone ash was dissolved in a few drops of concentrated nitric acid and diluted with distilled water so as to have approximately 1 mg of ash per ml solution. One ml of this diluted solution was taken in a centrifuge tube and 1.0 ml of water and 2-3 drops of indicator bromo cresol green were

added. A saturated solution of sodium acetate in water was then added to the tube till the solution turned blue. To this was added 3% oxalic acid till the solution turned yellowish green. The tube was kept in a sand bath for 45 min at 80° to allow complete precipitation of calcium oxalate. The precipitate was allowed to settle overnight, the sample centrifuged the following day and supernatant drained off.

The precipitate was washed with 3 ml of 2% ammonia solution, again centrifuged and the supernatant drained off. This was repeated till the supernatant was colourless. The precipitate obtained was dissolved in 3 ml of 1N sulfuric acid, kept at 80° for 3 min and titrated against 0.01N potassium permanganate. Calcium content was calculated by assuming that a titre reading of 1 ml of 0.01N $KMnO_4$ is equivalent to 2 mg calcium.

Phosphorus : The method described by Fiske and Subbarao (1925) was used. One ml of the sample prepared for the estimation of calcium was diluted to 20 ml with distilled water and 1 ml of this further diluted to 8.6 ml with water. To this was added 1 ml of molybdate II reagent and 0.4 ml of aminonaphthosulfonic acid (ANSA). The contents of the tube were mixed well on a vortex mixer and allowed to stand at 27-30° for 30 min and the colour developed read at 660 mμ on a Klett Summerson Colorimeter against a blank containing 8.6 ml glass distilled water, 1 ml molybdate II and 0.4 ml ANSA. A

standard graph was obtained using aliquots of a standard phosphorus solution containing 4 to 20 µg of phosphorus. Bone phosphorus was calculated therefrom.

Nitrogen balance

Collection of samples : Animals were transferred to stainless steel metabolic cages for balance studies. Food intake was recorded carefully and urine and feces collected for 3 days unless stated otherwise. Body weights were taken at the beginning and end of the study. Urine was collected in bottles containing 5 ml of toluene. Funnels were used to avoid spillage. Feces were collected separately.

For the studies during gestation, the samples were collected during days 14 to 17 of gestation.

The collection of samples during lactation posed a problem because it was neither possible to separate the pups from their mothers for long periods without affecting their growth nor was it possible to allow them to stay in the metabolic cages without allowing their urine and feces to get mixed up with those of the mother's. This problem was sought to be solved by staggering the collection over a six day period so as to get a 24 h collection at the end. Mothers were kept in the metabolic cages alone for 4 h a day for six consecutive days to obtain a 24 h sample. Errors due to diurnal variation were minimized by spreading the six 4-hourly collections around

the clock, e.g. on day 1, collections were made from 8 am to noon; on day 2, from 12 noon to 4 pm, on day 3, from 4 pm to 8 pm; and so on for six days. Collections were begun on the 12th day of lactation which is believed to mark the period of maximum milk production (Reddy et al, 1964; Redman and Sweney, 1976). This also enabled to complete the collection on day 18 when the consumption of the maternal diet by the pups is marked although negligible quantities are consumed from day 15 on wards.

Estimation of nitrogen : The method described by Hawk, Oser and Summerson (1954) was used. The volume of the urine collected was made up to 100 ml with distilled water. 10 ml of this solution was taken in a kjeldahl flask of capacity 250 ml. To this was added 10 ml of conc. sulfuric acid. A pinch of copper sulfate-potassium sulfate mixture was added as catalyst. The mixture was allowed to boil in a digestion chamber till it became light green (This happened in roughly 1 h) and the boiling was continued for another hour.

After cooling to 27-30^{0} the contents of the flask were diluted and the volume made up to 100 ml with water. Ten ml of 2% boric acid solution containing 1-2 g drops of mixed indicator were placed in a conical flask. A known amount of the digested sample (2.5-5.0 ml) was quantitatively transferred to the chamber of the steam distillation apparatus, cleaned of any contaminating ammonia by a blank distillation, Ten ml of

50% sodium hydroxide were then added and the generation of steam in the boiler was started. Simultaneously, the conical flask was arranged so that the tips of the condensor outlet dipped below the surface of the solution. The sample was steam-distilled for 3 minutes after the boric acid solution became green. The ammonia content of the distillate was determined by titrating against 0.01N sulfuric acid. Total nitrogen content was calculated by assuming that a titre reading of 1 ml of 0.01N H_2SO_4 is equivalent to 0.28 mg nitrogen.

An essentially similar procedure was followed in the case of feces and food except that the three day collection (feces) or 5 g aliquot (food) was taken in toto and digested with 25 ml of conc. sulfuric acid for 4-5 hours.

Serum vitamin A : The method of Neeld and Pearson (1963) was used. To 0.2 ml of serum were added 0.2 ml distilled alcohol and 0.8 ml petroleum ether (60°-80° grade). The contents were mixed thoroughly and centrifuged. The upper phase was transferred to another tube and vacuum dried. The residue was taken up in 0.25 ml chloroform. 0.05 ml of this was transferred to a microcuvette and 0.6 ml of trifluoroacetic acid reagent added. The solution was read exactly after 30 sec. at 660 mμ in a Beckman Spectrophotometer using the microattachment against a blank containing 0.05 ml chloroform and 0.6 ml trifluoro-acetic acid mixture. A standard graph was obtained using

aliquots varying from 5 i.u. to 20 i.u. of vitamin A acetate and serum vitamin A calculated therefrom.

Details of the reagents and standards used are given in Table 20.

Behavioral studies

Open field activity :- The method described by Whimbley and Denenberg (1967) was used. For this purpose, the base of a large, black wooden box of dimensions 116 cm x 116 cm x 35 cm was divided into 25 equal squares with white lines.

Procedure :- The animals were starved overnight and tested in the morning in a room maintained at 27-30°. The animal was placed in one corner of the box and its activity, noted for the next 3 minutes in terms of the number of squares traversed and standing up reactions. Crossing the square with the fore paws alone was taken as a score. Standing on the hind limbs supported by the forepaws or otherwise was taken as a standing up reaction.

Each animal was given one trial per day for six consecutive days and the average score for the six day period was computed.

Activity in an activity wheel (Experiment 1b)

Apparatus :- A drum of diameter 25 cm and height 16 cm which can freely rotate without obstruction on its axis was used. The flat ends of the drum were made of wood while the

Table 20 : Details of reagents and standards used.

Reagent	Method of preparation
aminonapthosulfonic acid (ANSA)	195 ml of 15% sodium bisulfite solution taken in a 250 ml glass stoppered cylinder, 0.5 g ANSA and 5 ml of 20% sodium sulfite added and the contents shaken well so as to dissolve the ingredients and the solution transferred to a brown glass bottle and stored at 0-4°C
1% ammonia	1 ml of 25% ammonia (wt/wt) diluted to 100 ml with water.
2% ammonia	2 ml of 25% ammonia (wt/wt) diluted to 100 ml with water.
biuret reagent (stock)	22.5 g rochelle's salt (sodium potassium tartrate) dissolved in 200 ml of N/5 NaOH. 7.5 g copper sulfate (hydrous) added. 2.5 g potassium iodide added to this solution and the volume made up to 500 ml with N/5 sodium hydroxide and stored in an amber coloured bottle at room temperature (27-30°C).
biuret reagent (working)	20 ml of stock biuret reagent diluted to 100 ml with N/5 sodium hydroxide containing 5 g of potassium iodide per litre.
2% boric acid	2 g boric acid dissolved in water and the volume made upto 100 ml with water.
bovine serum albumin standard	40 mg bovine serum albumin dissolved in10 ml water and 1 ml of this further diluted to 10 ml with water so as to get a concentration of 400 ug/ml.
bromo cresol green indicator	1 g of bromo cresol green dissolved in100 ml distilled ethanol.
cholesterol standard	100 mg cholesterol dissolved in100 ml distilled ethanol and 4 ml of the same diluted to 100 ml with ethanol so as to get a concentration of 40 ug/ml.

contd...

Table 20 : contd.

Reagent	Method of preparation
chloroform : methanol mixture	200 ml chloroform mixed with 100 ml methanol, shaken well and stored in dark bottles at 10°C.
copper sulfate - potassium sulfate dry mixture	crystals of copper sulfate and potassium sulfate mixed in the ratio of approximately 1:10.
ethanol distilled	commercial alcohol distilled at 79°C.
Folin ciocalteau reagent	A mixture of 100 g sodium tungstate, 25 g sodium molybdate, 700 ml 85% phosphoric acid and 100 ml concentrated hydrochloric acid refluxed on a sand bath at about 150°C using an air condensor in a 1.5 litre flask for about 18 hrs. 150 g lithium sulfate, 50 ml water and a few drops of bromine water added and the mixture boiled over a sand bath for 15 min. without the condensor to remove excess bromine, cooled to room temperature (27-30°) and the volume made up to 1 litre with water, filtered through glass if necessary, stored in an amber coloured bottle at 10°C and diluted 1:2 with water before use.
iron reagent	2.5 ferric chloride dissolved in100 ml of 87% phosphoric acid and 8 ml of this solution diluted to 100 ml with concentrated sulfuric acid.
lowry's A	2 g sodium carbonate dissolved in 100 ml of 0.1N sodium hydroxide.
lowry's B	100cg of sodium potassium tartrate dissolved in water, the volume made up to 10 ml and 50 mg copper sulfate dissolved in this solution.
lowry's C	50 ml of lowry's A mixed with 1 ml of lowry's B, the mixture being prepared fresh each time.
mixed indicator	25 mg of bromo cresol green and 75 mg of methyl red dissolved in 100 ml ethanol.

contd...

Table 20 : contd.

Reagent	Method of preparation
molybdate II	25 g of ammonium molybdate dissolved in 200 ml of water, 300 ml of 10 N sulfuric acid added and the mixture diluted to 1000 ml with distilled water.
3% oxalic acid	3 g of oxalic acid dissolved in water and volume made upto 100 ml.
phosphorus standard	35 mg monopotassium phosphate dissolved in a minimum amount of water and transferred to a 100 ml volumetric flask. Volume made up to 100 ml with water containing 1 ml of 10 N sulfuric acid per 100 ml, to get a concentration of 80 µg/ml 1 ml of the same diluted to 10 ml with water to get working standards.
0.01 N potassium permanganate	1.58 g of potassium permanganate dissolved in water and the volume made up to 1000 ml.
scintillation liquid	6 g of 2-5 diphenyl oxazole (PPO) and 400 mg of 1.4 bis-5 phenyl oxazole-2-ylyl-benzene (POPOP) dissolved in toluene and the volume made up to 1 litre.
0.02 N sodium hydroxide	0.08 g of sodium hydroxide pellets dissolved in water and the volume made up to 100 ml.
0.1 N sodium hydroxide	0.4 g of sodium hydroxide pellets dissolved in water and the volume made up to 100 ml.
0.2 N sodium hydroxide	0.8 g sodium hydroxide pellets dissolved in water and the volume made up to 100 ml.
N sodium hydroxide	4 g of sodium hydroxide pellets dissolved in water and the volume made up to 100 ml.
40% sodium hydroxide	40 g of sodium hydroxide (commercial flakes) dissolved in water and the volume made upto 100 ml.

contd...

Table 20 : contd.

Reagent	Method of preparation
sodium acetate (saturated)	sodium acetate crystals added to 100 ml of water in increasing quantities with continuous stirring till undissolved crystals were seen to settle at bottom.
solvent ether ethyl alcohol mixture	solvent ether and alcohol mixed in the proportion of 1/1 : : v/v.
standard serum	serum of known nitrogen and protein content as determined on suitable aliquots preserved in the deep freeze.
sulfate-sulfite reagent	20.8 g of sodium sulfate and 7.0 sodium sulfite dissolved in dilute sulfuric acid (prepared by adding 0.2 ml concentrated sulfuric acid to 90 ml of water) and the volume made up to 100 ml with water.
0.02 N sulfuric acid	1.7 ml of concentrated sulfuric acid (36 N) made up to 3 litres with water.
1 N sulfuric acid	10 ml concentrated sulfuric acid mixed with water and the volume make up to 360 ml.
10% trichloroacetic acid	10 g of trichloroacetic acid dissolved in water and the volume made up to 100 ml.
trifluoroacetic acid reagent	1 volume of trifluoroacetic acid mixed with 2 volumes of chloroform (this was prepared as required and not stored for more than 2 hrs at 27-30^{o}C).
vitamin A standard	3.44 mg vitamin A acetate dissolved in chloroform and the volume made up to 50 ml with chloroform (this was prepared as required and stored at no more than 36 hrs) at less than 10^{o}C.

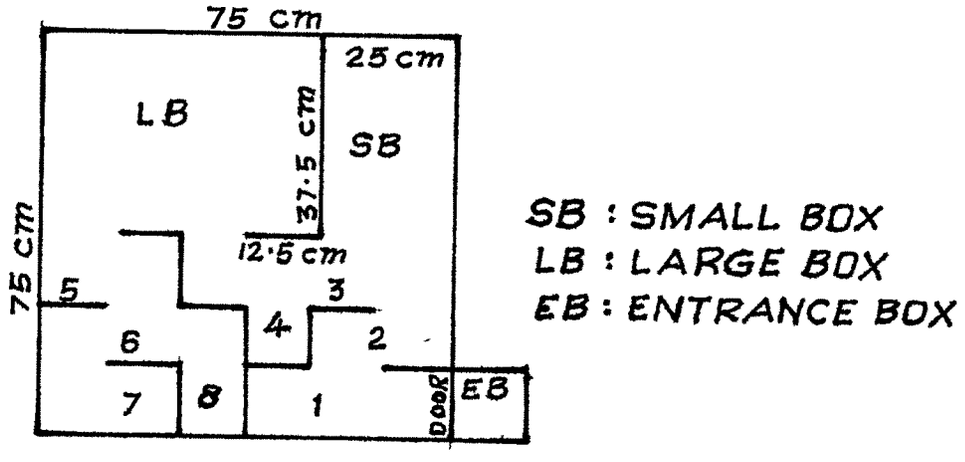
Water and alcohol were glass distilled before use.

curved surface was made of wire mesh. One of the flat ends had an entrance door measuring 10 cm x 10 cm.

Procedure :- The animals were started overnight and tested in the morning. The animal was placed in the wheel through the side door made for the purpose. The movement of the animal caused rotation of the wheel on its axis. A micro-switch attached to one of the supports of the wheel aided in measuring the number of rotations made. However, the wheel was found to make several half and quarter rotations. Hence one rotation of the drum was subdivided into eight segments by chalk markings on the wheel and the rotation recorded as one or a fraction thereof according to the turn of the wheel. Standing up reactions and time spent in grooming were also recorded.

Maternal behavior (Experiment 2a):- The method of Frankova (1974) was used with minor modifications.

Apparatus :- A maze box 75 cm x 75 cm x 30 cm with opaque walls and a transparent top was divided into sectors as shown in the figure.



Procedure :- Day 1 : The pups were distributed in sectors 1-8 and the mother put in the entrance box (EB) for 2 minutes. The door between the entrance box and the maze was then opened and the mother allowed to enter the maze. The exploratory activity of the mother and her contacts with the pups were recorded. Contacts with the pups included shifting, + touching, licking, retrieving or nursing the pups. The total time spent by the mother away from the pups in exploring her surroundings was designated exploratory activity. The time that elapsed before she made any contact with the pups was designated 'latency'. Retrieval of the pups consisted of shifting the pup from one place to another of apparently greater safety.

Day 2, trial (1) :

The floor of the small box (SB) was covered with nesting material. The mother and all her pups were placed in the small box and left undisturbed for the next 10 min. Activities were recorded as on Day 1.

Day 2, Trial (2) : The mother was placed in the entrance box (EB) for two minutes and the entrance to the small box opened. The behavior of the mother in the maze was recorded for the next 20 minutes as on Day 1.

Other measures :-

Growth :- Body weight gain in one week was taken as the index of growth.

Efficiency of feed utilization

Body weight gained per gram food intake was taken as a crude index of the efficiency with which food is utilized for tissue production. To measure food intake, weighed quantities of food were given on day 1. Equivalent quantities were dried in a hot air oven at 60^{0} - 70^{0} to obtain dry weights of food consumed. On day 2, food leftover in the container and that spilt in the tray was collected, dried and weighed.

Reproductive performance

The efficiency of reproductive performance was sought to be measured using as criteria maternal weight change during gestation and lactation, maternal food intake during gestation and lactation, the efficiency of feed utilization for tissue growth (maternal and fetal), the number of pups born, the number surviving at weaning, the per cent mortality of progeny and the growth rate as judged by body weights till weaning.