Research investigations carried out during the year at National Institute of Nutrition (NIN), Food and Drug Toxicology Research Centre and National Centre for Laboratory Animal Sciences at Hyderabad and National Nutrition Monitoring Bureau (NNMB) revealed new findings pertaining to several aspects of nutrition science. The NNMB had conducted a series of surveys in different parts of the country. Over 10,000 households were surveyed in 530 villages from nine states to determine the nutritional status of population (as a part of 54th round of consumption-expenditure survey). The data on food and nutrient intakes of individuals analysed during the year indicated that micronutrient intake among people was extremely inadequate and especially children of pre-school age were seen consuming diets bereft of essential micronutrients. However, in comparison with the nutrient intake data of 1975-79, the data collected in 1996-97 revealed a significant improvement in the fat, energy and vitamin A intake among population groups. Other areas of study included food chemistry, food and drug toxicology, nutrition and infection and nutrition in the elderly.

COMMUNITY STUDIES

Diet and Nutritional Status of Rural Population

The community studies continued to place emphasis on the assessment of diet and nutritional status of the population. This year, the investigations included, (i) monitoring of nutritional status of people in rural areas in 9 States coming under NNMB, (ii) district level nutrition profile surveys in Uttar Pradesh and West Bengal, (iii) drought surveys in the States of Andhra Pradesh, Gujarat and Rajasthan, (iv) micronutrient supplement trials on residential school children to assess the influence on cognitive performance and growth of children, (v) assessment of the current status of Integrated Child Development Services (ICDS) under Andhra Pradesh Economic Restructuring (APER) Programme in tribal and rural areas, and (vi) analysis of association between nutritional status and development indicators.

Out of the targeted 720 villages in nine states, 14,400 households were selected by NNMB for the assessment of nutritional status of the people. During this year, 10,600 households from 530 villages were surveyed. The rest of the households in the remaining villages will be surveyed during 2001. Analysis of the individual dietary intakes, collected by the NNMB as a part of 2nd repeat survey, using 24-hour recall method revealed that about 13-55% individuals in different ages consumed recommended dietary allowance (RDI) for energy whereas the consumption of micronutrients was woefully inadequate, 50-70% of individuals consumed less than 30% of RDI for vitamin A especially the pre-school children had higher deficits in their diets. A comparison of the median individual intakes between 1975-79 and 1996-97 indicated that there was significant improvement in the intakes of fat, energy and vitamin A. The consumption was higher in all the age groups except 1-3 yr. old children during 1996-97. However, iron intakes were higher only among adults.

As part of district level nutrition profile survey, a study of diet and nutritional status was conducted in 80 districts in Uttar Pradesh (63) and West Bengal (17) in collaboration with the Institute of Applied Statistics and Development Studies, Lucknow and Child in Need Institute, Kolkata. So far, the Institute has contributed to district level database on diet and nutritional status of 170 districts from Assam, Haryana, Himachal Pradesh, Orissa, Punjab, Uttar Pradesh and West Bengal.

The drought surveys undertaken in Andhra Pradesh, Gujarat and Rajasthan revealed that the impact of drought was more severe in the state of Rajasthan (Fig.16). In general, a large number of households experienced food insecurity. However, no starvation (energy intakes <500 kcal) was reported in any of the households in all the 3 states. The prevalence of
undernutrition was by and large comparable to pre-drought surveys. It appeared that relief measures, particularly public distribution system, helped the drought-affected communities to a large extent in the prevention of severe forms of undernutrition.

The survey in ICDS projects in tribal areas of Andhra Pradesh revealed that though more than 75% of the beneficiaries availed the ICDS services, the proportion of 6-35 month old children participating in supplementary feeding was very low (20%) as compared to 48% in older children. Despite better performance with respect to immunisation of children against six childhood diseases or tetanus toxoid immunisation in pregnant women, the coverage under nutrition related services like distribution of iron and folic acid tablets and vitamin A was not adequate. Growth monitoring was not regular. These results point out to the need for strengthening growth monitoring and nutrition services in ICDS.

Repeat surveys carried out by NNMB have shown that though the dietary intakes remain unchanged, the nutritional status (as assessed by nutritional anthropometry) registered a change. Hence, a detailed statistical analysis was carried out to assess the relationship between data on diet and nutritional status and socio-economic and developmental indicators collected at the village level as part of National Council for Applied Economic Research (NCAER) - NNMB linked surveys. The results indicted that family size of more than six, protected water, SC/ST community status and low vitamin A intake were associated with prevalence of undernutrition.

**Operational Evaluation of the Stability of Iodine in Double Fortified Salt**

The NIN has successfully developed a formulation of double fortified salt (DFS, containing both iron and iodine) comprising common salt, ferrous salt, potassium iodate and sodium hexametaphosphate. The salt was considered stable at the laboratory and community levels. However, in a recently concluded residential school study at NIN, the iodine stability was found to be very poor in the DFS. It was observed that in some batches the quality of common salt was not up to the mark due to high magnesium content. The distribution of iron and iodine was not uniform.

**Fig. 16** Distribution of preschool children according to weight for height.
in some batches due to improper mixing at the time of production. An expert committee on DFS constituted by the ICMR has recommended a multicentric study to assess the stability of iodine in DFS using different types of salt for double fortification. Large scale production of the DFS and iodised salt is to be done at a factory in Chennai.

**NUTRITION AND INFECTION**

**Safety and Feasibility of Administering Vitamin A alongwith Oral Polio Vaccine**

In view of the persistence of vitamin A deficiency in children as a public health problem, the State Government of Orissa had launched a programme of administering massive oral dose (200,000 IU) of vitamin A alongwith OPV on the first National Immunisation Day of Intensive Pulse Polio Immunisation Programme to children between 12-42 months of age on 24th October, 1999. The feasibility and safety of this strategy was assessed by conducting a rapid post-immunisation survey in 5 randomly selected districts of Orissa on a total of 879 under-5 children, in collaboration with WHO and UNICEF. There was over 90% coverage of the target age group. Not a single infant below the age of 6 months received the dose. Nevertheless, there was a possibility of administering the dose to infants below the age of 12 months. Symptoms of indisposition, observed within 48 h of administration of the dose were not significant suggesting that vitamin A administration along with OPV is safe and feasible. However, training the health functionaries to administer the dose only to the specified age group is suggested.

**Nutrition-Infection Interactions in Acute Bacterial Meningitis in Children**

Acute bacterial meningitis is one of the major causes of morbidity in young children. The effect of nutritional status, microbial causes and cytokine profile on the outcome was investigated in 140 children hospitalised for acute bacterial meningitis. Malnutrition and presence of tumour necrosis factor (TNF) in cerebrospinal fluid was found to be significantly associated with adverse outcome (survival with permanent neurological sequelae or death).

**MICRONUTRIENTS AND TRACE ELEMENTS**

**Fortification of Sugar with Micronutrients**

During this year, stability of fortified sugars -one fortified with single nutrient like iron or iodine and the other fortified with both iron and iodine, was tested. The stability of iodine as well as iron was found to be very good in the sugar fortified with a single nutrient. At the end of 6 months, iodine stability was not uniform with different chemical sources of iron tested. Although the stability of iodine was good with ferrous glycinate, about 50% loss occurred with ferrous sulphate or ferrous fumarate.

**Bioavailability of Iron**

To answer a query from Human Rights Commission, bioavailability of iron from the pickles commonly consumed in combination with cooked rice and phulka or chapati was assessed. The total iron content of pickles in combination with rice was 19 mg and that with phulka was 103 mg/g. Addition of five pickles to cooked rice decreased the *in vitro* bioavailability of iron in the case of four of them from 32% to 14-23%. However, in combination with wheat-based phulka, the bioavailability increased from 4.2% to about 7% with three of the five pickles. With other pickles there was no change in bioavailability.

**Iron Availability from Ayurvedic Hematinic**

A new hematinic, containing a blend of ayurvedic formulation, was tested for the management of anaemia. Besides iron, this preparation contained herbs presumed to enhance the bioavailability of iron. The relative bioavailability of iron from this preparation was found to be only 66.7% as against one reference preparation used to regenerate haemoglobin in iron depleted rats. The efficacy of this preparation as a hematinic needs to be tested in humans.

**Iron Absorption Promoters in Wheat Flour**

Fortification of wheat flour with iron and other absorption promoters was attempted to enhance the bioavailability of both exogenous and endogenous dietary iron. Incorporating iron absorption promoters such as ascorbic acid and EDTA in wheat flour at a molar ratio of 1:1 was found to enhance the *in vitro* availability of the endogenous and exogenous iron respectively.
Studies on Selenium

Selenium nutritional status was assessed based on the body mass index, with the cut off level at 18.5. Both plasma selenium and a functional parameter viz. the activity of red cell glutathione peroxidase were estimated. While plasma selenium levels were significantly reduced in undernourished and anaemic subjects, activity of glutathione peroxidase was not affected.

Bioavailability of selenium from cereals like rice, wheat, sorghum and pulses was carried out by standard in vitro methodology simulating gastric conditions. While initial digestion with pepsin showed the level of available selenium varying between 32% and 72%, it was around 75% after second digestion with intestinal enzymes.

DIET AND NON-COMMUNICABLE DISEASES

Biochemical and Metabolic Studies with Sesame Lignans

The beneficial effects of antioxidants in prevention of diet-related chronic diseases are being increasingly emphasised. Non-glyceride fraction of certain oils contains natural antioxidants, such as tocotrienols and carotenes (palm oil, red palm oil), oryzanol and tocotrienols (rice bran oil) and lignans namely, sesamol (S1), sesamin (S2) and sesamolin (S3) (sesame oil). Lignans are known to impart greater stability to sesame oil. Studies were initiated to determine the heat stability of sesame oil in combination with other oils (palm oil and groundnut oil). Heating of single oils (sesame, groundnut and palm) resulted in time-dependent decrease of total tocol content. Percentage retention of total tocols (tocopherols and tocotrienols) at the end of 30 min. (65-69%), 1 h (42-50%) and 2 h (17-20%) of heating was similar in all the three oils. Sesame in combination with palm oil showed higher retention of total tocols as compared to either of the single oils up to 2 h heating. However, sesame oil in combination with groundnut oil did not increase retention of total tocols. The decrease in total tocols in heated oils was associated with increase in peroxide values and TBARS levels. Heating of sesame oil or its blends was associated with decrease in total lignans (30-40%) which was due to decomposition of sesamolin. However, sesamol which has higher antioxidant activity than sesamolin, increased from negligible levels to ~0.04 g/kg oil. These findings suggest that sesame blend with palm oil may be more stable to oxidative deterioration due to heating as compared to single oil.

Effect of Dietary Trans Fatty Acids on Insulin Resistance and Function of Adipocytes

Insulin resistance is an important risk factor associated with type II diabetes, chronic heart disease and obesity. Adipose tissue and skeletal muscle are the major target tissues for insulin action.

Recent studies have shown that there is a positive correlation between skeletal muscle and adipose tissue plasma membrane PUFA composition and insulin action. Since Indian vanaspati contains high levels of trans fatty acids, a study was conducted to investigate the effect of dietary trans fatty acids (vanaspati) at different levels of linoleic acid on adipocyte and skeletal muscle lipid composition, fluidity and function. The results showed that a casein-based diet, which provided 22 en% fat, 3 en% trans fatty acids, significantly altered the plasma triglyceride and glucose levels, adipose tissue and skeletal PUFA composition and adipose tissue plasma membrane fluidity. These changes were observed at both the levels of linoleic acid (2 en% and 4 en%) in the diet. The results further show that the response to vanaspati on PUFA metabolism may vary from tissue to tissue. Studies on the effects of dietary trans fatty acids on functional properties of adipose tissue and skeletal muscle such as glucose transport, insulin binding and lipolytic activity are in progress.

Antioxidant Status in Geriatric Population

Many degenerative diseases occurring in old age have been attributed to free radical associated damage to the tissues and a reduction in antioxidant status. In order to understand the level of lipid peroxidation and the activity of a key antioxidant enzyme, glutathione peroxidase (GSHPx), a study was undertaken in different age groups (40 yr to >60 yr). The study revealed that as age advances, GSHPx tends to decrease with a concomitant elevation in malondialdehyde (MDA) which could be the underlying mechanism for free radical-mediated degenerative diseases in old age.

FOOD CHEMISTRY

Effect of Fly Ash on Nutritional Quality of Foods

Fly ash is a by-product of thermal power stations and its disposal is posing a gigantic problem in recent years. In view of this, on the request of Fly Ash Mission, a detailed study was undertaken on the effects of fly ash on nutritional quality of foods.
Major ICMR Research Projects in Nutrition
ash on nutritional quality of foods such as rice, wheat, peas, mustard, groundnuts and vegetables. Proximate composition of these food items grown in soils conditioned with fly ash did not show any definite or specific changes attributable to fly ash. In fact, foods grown on fly ash have shown occasional beneficial effect with respect to protein or fat in some foods. Trace minerals and heavy metal content of foods was essentially similar whether grown on soils with or without fly ash. Similarly, biological evaluation did not show any differences in animals, which were fed foods grown on soils with or without fly ash.

**PATHOLOGY**

**Effect of Food Protein and Vitamin Restriction on Apoptosis in Intestinal Mucosa**

The effect of 50% food, vitamin and protein restriction was studied in small intestinal mucosal cell apoptosis in rats for a period of 20 weeks. Body weights, haemoglobin and serum protein levels significantly decreased on 50% diet restriction and 75% protein restriction while apoptotic counts increased in the terminal portions of the villi in 50% vitamin and 75% protein-restricted groups.

**FOOD AND DRUG TOXICOLOGY**

**Dietary Intake of Aflatoxins and Organochlorine Pesticides**

A study has been undertaken in a rural area to assess the dietary intake of aflatoxins and organochlorine pesticide residues. Nineteen 24-hour diets, consumed by adult men, were collected from 2 villages near Hyderabad, and analysed for pesticides and aflatoxins. The results of the study indicated that their levels were very low and may not pose a risk to consumer health.

**Assessment of Dietary Intake of Synthetic Food Colours**

About 315 food items were analysed including sweet meats, vegetables, snacks, chutneys, bakery foods, sherbets, soups and biryani to determine the kind and the quantity of food colours added to them. 95% of these foodstuffs were found to contain permitted food colours while 5% used non-permitted colours. However, 31% food items in the permitted colours category contained colours within the permissible limit of 100 ppm, while 69% had colours above the prescribed limit.

Certain beverages and sweet meats contained as high as 9.4 mg/ml of colours. Pre-schoolers and school-going children were found to be consuming excessive amounts of the permitted colours sunset yellow and tartrazine mainly through sherbets and confectionery items.

**Application of Hazard Analysis Critical Control Point (HACCP) to Animal Products**

Foods of animal origin pose a risk of transmission of zoonotic and human diseases. Poultry and shrimps were studied to explore the possibility of using HACCP in minimising the risk of disease. All the 34 samples of chicken meat contained aerobic bacteria, the counts ranging from $10^3$ to $10^7$ cfu/g. In these samples, 88% had coliforms, 76% staphylococcus and 53% salmonella. Nine out of 18 samples of chicken biryani also had aerobic bacteria. In addition, all the samples of shrimps sold at 35 outlets in Hyderabad contained aerobic bacteria, the counts ranging from $10^7$ to $10^8$ cfu/g.

**Biotechnological Approach to render Mouldy Sorghum Safe for Consumption**

Sorghum is highly susceptible to mouldy damage during unfavourable weather conditions. Such damage affects the yield and the quality of the grain, decreases the nutritive value and favours the production of mycotoxins. A study was carried out using microorganisms like lactic acid bacteria through natural fermentation to detoxify contaminated sorghum and then to convert it into a value-added animal feed ingredient.

**NUTRITION AND CANCER**

**Nitrosamines**

Nitrosamines have been postulated to play a role in the etiology of human stomach cancers. Nitrosation of amines *in situ* and identifying the populations at risk was taken up for study this year. In addition, the effect of inhibitors of nitrosation under *in vivo* condition was also studied. The study revealed that ingestion of turmeric could inhibit nitrosation *in situ* to the extent of 60-80%.

**Triglycerides, Insulin Resistance and Breast Cancer**

Elevation in fasting levels of triglycerides (TG) is associated with increased risk of breast cancer. Since
elevated levels of triglycerides and insulin resistance are closely related. A study was undertaken this year to understand the association of TG, HDL, and insulin resistance. Results indicate higher TG and lower HDL levels in breast cancer cases while there seems to be no relationship of these parameters to insulin levels.

OTHER STUDIES

Studies on Obese Mutant Rats

Research studies, covering both basic and applied aspects, were conducted on obese mutant rats (WNIN/Ob and GR-Ob), isolated and established at the Centre. Molecular analysis of the obese mutant rats concentrated on two aspects, viz. to identify the molecular lesion with reference to the known obese gene(s) in rodents and to establish DNA fingerprint specific to these mutants. As regards mutational change(s) in known obese rodent genes, it is now confirmed that the leptin gene in these animals has not altered. This is based on the analysis of the complete leptin gene sequence from (+/+) and (-/-) phenotypes. But, in the obese mutants, there appears to be a difference in its leptin receptor gene. The (-/-) phenotype showed an altered PCR product (devoid of 200 bp) compared to lean control (+/+). This is further confirmed by the leptin receptor binding data of the brain membrane preparations from these rats. A significantly low or decreased binding of $^{125}$I-labelled leptin was seen in homozygous obese (-/-) animals compared to lean control (+/+).

With reference to PCR based DNA fingerprinting studies using random primers, a male specific PCR product from obese males was obtained. Efforts are now on to clone this product in a suitable vector and sequence the same.

Accelerated ageing in obese mutant rats as indicated by free radical damage to lipids and protein (as early as 35 days) was reported last year. This year, the study was extended to anti-oxidant enzymes like catalase, glutathione peroxidase, glutathione S-oxidase and superoxide dismutase. These enzymes showed differences in the obese rats, generally around 90 days, but in some cases around 35 days itself (for eg. glutathione peroxidase in kidney and heart). Significant changes in these enzymes reflect decreased antioxidant defence in the mutant rats.

Cogent-db is an herbal drug consisting of nine herbs with neem, turmeric, amla and Jamun as major constituents. This drug was earlier tested by others in alloxan-diabetic rats and diabetic patients, and is currently available in the country across the counter. The anti-diabetic claim of this drug was verified using GR-Ob rats and it was found that the drug was effective at a dosage of 100mg/kg body weight. The drug brought about a significant reduction in cholesterol and triglycerides. These effects continued even one month after the withdrawal of the drug and all the animals in the treated group showed vigorous physical activity.