Proc. Indian natn. Sci. Acad. **B57** No. 6 pp. 361-368 (1991)

J C BOSE MEDAL LECTURE 1989

Genetic Consequences of Inbreeding in a Large Human Population

N APPAJI RAO

Department of Biochemistry, Indian Institute of Science, Bangalore 560 012

(Delivered on 1 January 1990)

Inbreeding in human populations has fascinated sociologists and geneticists from a long long time. Intuitively, we expect that recessive traits occur with increased frequency in the progeny of consanguineously related parents as they have a greater chance of inheriting a mutant gene from a common parent. Two individuals are said to be consanguineously related if they have atleast one common ancestor. The common ancestor must not be too remote or else the concept becomes meaningless. For convenience, in human genetics, ancestors more remote than great-great grand parents are not considered significant. Progeny of consanguineous parents are termed inbred. Genetic effects of inbreeding can be traced to the fact that an individual may carry a mutant gene inherited from a common ancestor. The modes of inheritance of these genetic disorders are classified as autosomal recessive, where a child inherits one mutant gene from each of the parent and is affected by the disorder; whereas the parents who have only one copy of the mutant gene are apparently normal. In autosomal dominant inheritance even if one copy of the gene is mutated the individual is affected by the disorder. In the X-linked recessive inheritance, as the females carry two copies of the X-chromosome, if one allele is mutated, they function as carriers and are apparently normal. As the males, have only one X-chromosome, if the gene is mutated he is affected by the disorder. In other words, females are carriers and transmit the mutant gene with 50% frequency to male offsprings who are affected by the disease.

One of the earliest studies on the effects of inbreeding was that of Darwin (who was himself an offspring of a consanguineous marriage), who studied the proportion of first cousins offspring among Oxford and Cambridge boating men and showed that there was 2.4% higher number of them among the non-consanguineous group (Darwin 1875). While endowed with great charm, these results of Darwin cannot be used to define the nature and extent of human inbreeding effects in man. Garrod (1902) showed that of the 18 cases of the inborn error of metabolism-Alkaptonuria, diagnosed in Europe and North America, 12 had been born to parents who were first cousins. However, in order to maintain a sense of perspective on the level of morbidity, Garrod (1902) also reminded his readership of the extreme rarity of the condition among first cousins offspring as a whole, only 6 out of 50,000.

The practice of marrying close relatives is common among the populations of South India. Many text books, (Cavalli-Sforza & Bodmer 1971), on human genetics as well as Western social wisdom presume that consanguinity is confined mostly to areas of low population density and leads to decreased fertility, increased pre-reproductive loss, consequently to dwindling populations. Against this notion, in the 1981 census, the population of Karnataka, Andhra Pradesh, Tamil Nadu and Kerala, where consanguinity is extensively practiced, was 168 millions. It was suggested that continued inbreeding against a background of infectious diseases and malnutrition may have resulted in the elimination of deleterious genes from the population (Sanghvi 1966). My colleagues and myself therefore asked ourselves which of the two alternatives, (a) has

inbreeding led to increased incidence of genetic disorders or (b) to cleansing of the gene pool, is correct?

Before I discuss these questions let me indicate the consequences of an autosomal recessive disorder. Due to an alteration in the gene either by mutation or deletion or other reasons, the product when it happens to be an enzyme could become inactive. For example, in Phenylketonuria, a disease caused by the deficiency of phenylalanine hydroxylase, the following consequences arise. Phenylalanine, which is derived from the diet is normally metabolised to tyrosine which is required for a variety of important products such as melanin, a pigment in the skin; thyroxine and epinephrine, which are hormones or neurotransmitters. When the enzyme is inactive, phenylalanine accumulates and the increased levels are channeled into toxic products. The combined effect of the deprivation of the metabolites arising from tyrosine and the toxic effects of degradative products of phenylalanine will lead to an irreversible brain damage and mental retardation.

One method to examine the validity of one of the questions raised in an earlier paragraph is to examine for the mutant gene among the mentally retarded individuals. Prior to our study, only a few of disorders were identified in our population. It can be seen from the table 1 that several classical autosomal recessive disorders are present in the population as indicated by their occurrence among the mentally retarded individuals (Sridhara & Rama Rao et al. 1977).

As one of out objectives was to examine the effects of inbreeding in humans, we examined the coefficient of inbreeding (F) which indicates the "chance of an individual receiving a mutant gene from a common ancestor" and this value reflects the inbreeding level of the population. It was seen (table 2) that the inbreeding coefficient in the mentally retarded individuals with a single gene defect (0.08) was higher than in the general population (0.07) (Rao & Inbaraj 1979). In the American population where marriage among close relatives is frowned upon, it is 0.001, (Freire-Maia 1968) a very low value compared to that in South India (Rao & Inbaraj 1979). One obvious and hasty conclusion that could be drawn from these results was that inbreeding leads to increased occurrence of mental retardation and increased rate of genetic disorders in the population. A moment's reflection would indicate some of the fallacies in this conclusion. One such fallacy arises from the fact that the samples were drawn from a highly preselected group with a bias in the referrals. It was therefore, necessary to substantiate this conclusion by examining the general population.

One interesting aspect of these genetic disorders of amino acid metabolism is in that the severity can be ameliorated to almost complete recovery, in a few cases, by feeding the childern a special diet lacking a particular amino acid. For this purpose it is necessary to begin the treatment before the symptoms appear and within a few weeks of birth. As presymptomatic screening for genetic disorders of amino acid metabolism has been demonstrated to be feasible in the developing countries and these disorders have been demonstrated to be present in our population (Sridhara Rama Rao et al. 1971) and most important they can be ameliorated, prompted us to presymptomatically screen the newborn and examine the relationship between inbreeding and the incidence of genetic disorders.

In addition to collecting three drops of blood for amino acid analysis, trained volunteers interview the mother and obtain information on consanguinity, religion, number of liveborn and living children, etc. This information was coded and stored on a computer at the Indian Institute of Science, Bangalore, and analysed. The blood spot (0.6 cm diameter) was cut and eluted overnight into 50 µl of ethanol and the extract was subjected to thin layer chromatography. The amino acids were located by reaction with ninhydrin. The separation of the amino acids were very good and 13-15 discrete amino acid bands were obtained (Ireland & Read 1972). The elevated levels of amino acid showed up strikingly as a thick band on

the chromatogram indicating a possible genetic disorder. For example increased levels of phenylalanine is seen in phenylketonuria, tyrosine in tyrosinemia, leucine, isoleucine and valine in maple syrup urine disease, etc. Repeat analysis was done with the second spot. The child was followed up in more than 50% of the cases and confirmation of the disorder made biochemically (Appaji Rao N et al. 1988).

This programme was begun in 1980 and after initial teething trouble it has been going on steadily since 1982. The date (December 31, 1989) we have analysed more than 100,000 newborn children, drawn from 50 hospitals and Nursing Homes in Bangalore and Mysore (Appaji Rao et al. 1988).

Table 3 shows the type of pregnancies studied. There are a small number of twins and triplets and one quintuplet. Inbreeding contrary to expectation has had no effect on the twining rate which is lower for the Indian population compared to Western cohorts (Radha Rama Devi 1981).

We detected 6 cases of hyperphenylalanenemia, 17 of tyrosenemia, 11 of branched chain aminoacidemia also known as maple syrup disease and 3 of histidinemia. The incidence of these disorders ranges one per 6,000 to 35,000 births. Tyrosinemia was found to be transient in many of these children and probably reflected prematurity of the baby as this enzyme was induced at later part of gestation (table 4). Generalised amino acidemia is not a genetic disorder and was caused by several unknown factors like infection, malnutrition, etc., and acts as a good internal control to compare inbreeding defects and the methodologies used. The incidence of single gene defects of amino acid metabolism in our population is very similar to that seen in several Western countries of Europe and America and in Japan. In all these countries it needs to be emphasised that there is very little inbreeding (Freire-Maia 1968). Table 5 shows the inbreeding coefficient of the children affected by single-gene disorders, generalised amino acidaemia, in the newborn and the general population. The values are all very similar and it can be concluded that inbreeding as practiced in

Table 1 *Inborn errors of metabolism detected in a survey of* 1280 *mentally retarded childern**

Disorder	Number
Phenylkentonuria	14
Maple syrup urine	5
Hartnup's	4
Homocystinuria	2
Mucopolysccharidosis	3

**Sridhara Rama Rao et al.* 1977

Table 2 *Inbreeding coefficients among mentally retarded and general population*

Disorder	Inbreeding coefficient
Single gene: Mentally retarted	0.18
General population (Rao & Inbaraj 1979)	0.02
American population	0.001

Table 3 *Types of pregnancies surveyed in newborn screening programme (total* 112, 339)

Type	Number
Single	110,848
Twins	747
Triplets	11
Quintuplets	1

Table 4 *Amino acidemias detected among* 112, 369 *newborns screened*

Disorder	Number	One in
Phenylketonuria	6	18,728
Tyrosinaemia	18	6,243
Glycinaemia	7	26,053
MSUD	11	10,215
Histidinaemia	3	37,456
General amino acid amemia	70	1,605

Karnataka has probably had no large effect on the incidence of these genetic disorders.

It has been generally postulated that unbanization and break up of the joint family system would have resulted in decreased consanguinity. However, the data in table 6 showed 17% uncle-niece marriages, 11% first cousin and 2% second cousin and about 8% other unclassified relatives. This large number of inbred offspring in our study provided a very fascinating material to study the effects of inbreeding on human populations. I have already indicated the effects on genetic disorders and I shall now turn to two other parameters..

Table 7 shows the number of liveborn and living children born to more than 100,000 mothers, the ratio between the line born and the living indicates the proportion of survivors. In a standard text book on human population genetics it is mentioned that inbreeding leads to loss of fertility and increased prereproductive losses (Cavalli-Sforza & Bodmer 1971). An examination of the proportion of survivors indicated that the inbreeding effect if any was marginal as the value for all the groups was nearly similar. A detailed and statistical analysis carried out indicated that there was no significant difference among the groups (Bittles et al. 1985).

Table 8 shows the simplest of the statistical analyses, mean and the variance for the liveborn and the living. It is obvious from the figure that the mean number of childern was slightly different among the groups. The mean number of childern 2.2 shown in this table does not reflect the success of the family planning in Bangalore but indicates that the mothers are not at the end of the reproductive period. It was evident from these data that neither fertility (table 8) nor proportion of survivors (table 7) was seriously affected by inbreeding as practiced in this population (Bittles et al. 1985).

The classical formula of Morton (Morton et al. 1956) which relates the survivorship and the proportion of deaths due to genetic and environmental causes has been extensively used to assess the effect of inbreeding. It is obvious (figure. 1) that the points crowded close to the Y-axis were extrapolated assuming a linear relationship to indicate the effects of inbreeding. It is not necessary for me to highlight the hazards of such an extrapolation. Moreover, if we incorporate confidence limit of 5% into the data, the curve reaches ridiculous values when the inbreeding coefficient becomes large (Bittles & Makov 1985).

Bittles and Makov (1985) transformed this equation in different ways and analysed several models with different types of error distributions. An analysis of 20 different studies which have indicated strong inbreeding effects turn out to be mostly non-significant and many of the major ones become non-significant at 5% confidence limits in all the models.

One general conclusion that can be drawn from the study of the newborn is that considering the total population, inbreeding may have had no serious deleterious consequences. In human population studies or in any studies involving the human, the answers are not always black and white.

To illustrate this point I present the results of our study (Radha R D 1981) on sick children referred to us for diagnosing genetic disorders. Of the 404 cases examined, there were 35 single gene defects and we also saw a few chromosomal abnormalities and polygenic disorders (table 9). The predominance of single gene disorders probably indicates the bias of the referrals rather than any indication of true incidence. In view of our work on autosomal recessive disorders, there was a selection of patients suffering from these disorders. Small number of chromosomal abnormalities seen by us would indicate the preference of the clinicians to refer these patients to another centre in Bangalore. Table 10 shows the role of inbreeding in the etiology of these disorders. The inbreeding coefficient of this study group is slightly more than determined for the population as a whole. Although this observation could indicate the effect of inbreeding, it also might reflect the prejudice of the clinicians to suspect genetic disorders more often in consanguineous

Table 5 *Comparison of the inbreeding coefficient (F) among the newborns with single gene defects, other newborns and general newborns*

Group	F
Single amino acid defects	0.0336
General amino acidaemia	0.0350
General population (new born, Indian)	0.0298
American population	0.0010

Table 6 *Consanguinity profile of the parents of the newborns screened for amino acidaemias*

Class	Number	Percent
Nonconsangvineous	66,609	62.3
Uncle-Niece	18,240	17.1
First Cousin	12,157	11.4
Second Cousin	1,959	1.8
Others	4,454	4.1
Unknown	3,545	3.3

Table 7 *Number of live born and living children in the non consanguineous groups (Total:* 111,593)

Class	Liveborn	Living	Proportion
Nonconsanguineous	153,797	147,893	0.961
Uncle-Niece	44,417	42,537	0.957
First Cousin	29,721	28,450	0.957
Second Cousin	4,719	4,510	0.956
Other, unknown	22,281	20,903	0.940
Total	254,935	244,293	0.958

Table 8 *Mean and variance of live born and living children in relation to consanguinity*

	Live born		Living	
	Mean	Variance	Mean	Variance
Nonconsanguineous	2.20	±0.41	2.12	±0.38
Uncle-Niece	2.28	±0.61	2.18	±0.53
First Cousin	2.35	±0.66	2.25	±0.56
Second Cousin	2.32	±0.59	2.24	±0.53

Table 9 *Genetic disorders detected in* 404 *sick children*

Type	Number	Percent
Single gene		
Autosomal recessive	11	2.7
Autosomal dominant	24	5.9
X linked	5	1.2
Chromosomal	7	1.7
Polygenetic uncertain	16	5.6

Table 10 *Inbreeding trends in parents of children with genetic disorders*

Class	inbreeding (%)	Coefficient (F)
Total group	48.7	0.04
Single gene	45.2	0.05
Autosomal	60.9	0.06
General new born	32.2	0.03

Table 11 *Screening of newborn children* (2980) *for G-6-PD deficiency*

Sex	Deficient	Normal
Male	82	1437
Female	83	1378

Normal: 8-13 IU
Deficient: 0-6 IU

Table 12 *G6PD Sex ratio in other studies*

Place	Deficient (%)	
	Male	Female
Chandigarh	7.1	5.7
Jamnagar	22.6	14.1
Saudi Arabia	2.9	2.0
Bangalore	4.0	4.1

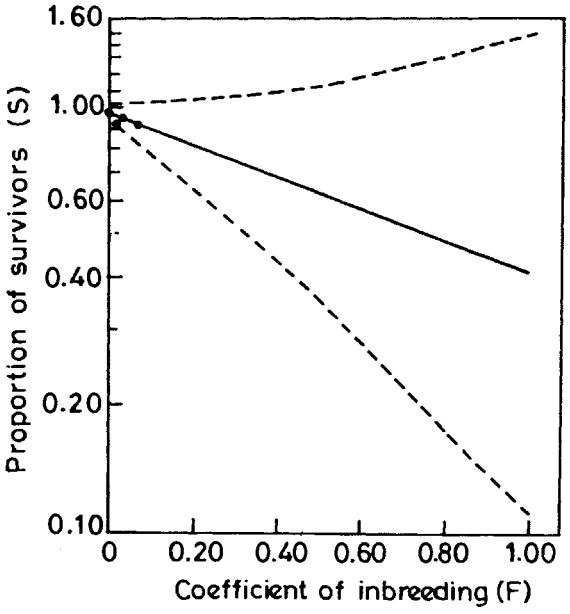

Figure 1

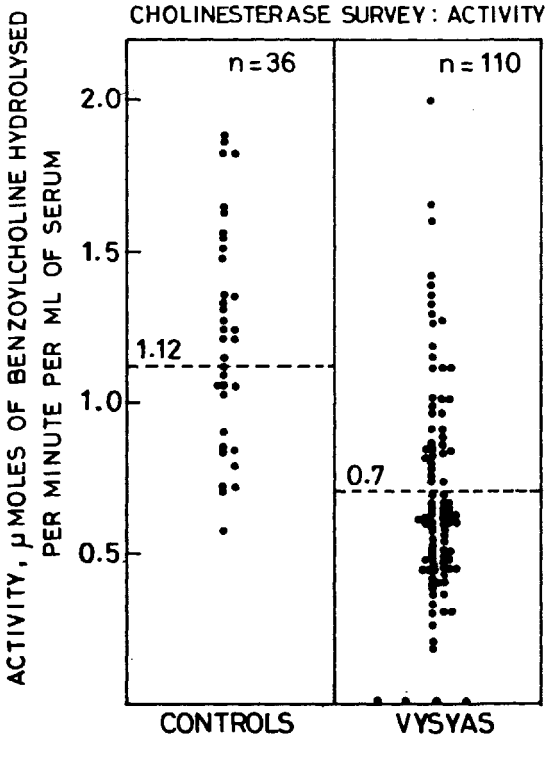

Figure 2

marriages than in other cases. This study confirms that many genetic disorders do exist in the population and that as environmental causes of childhood morbidity and mortality decline, the proportion of diseases with an exclusive or partial genetic etiology will increase. This phenomenon has been seen in many Western Societies.

The reemergence of malaria and the partial protection offered against this disease by glucose-6-phosphate dehydrogenase (G6PD) deficiency is offset by the drug-induced hemolytic anemia, these individuals suffer (Luzzatto 1985). G6PD is a useful polymorphic genetic marker and the preferred pattern of uncle-niece and maternal cousin marriages could lead to increased incidence of homozygosity in the X-chromosome. For these reasons, it was of interest to examine the incidence of G6PD deficiency in the newborn. One of the blood spots that were collected for amino acid screening was used to estimate the amount of G6PD. The spots were cut out and eluted into buffer and electrophoresed. After electrophoresis, the enzyme was located on cellulose acetate membrane by activities staining. G6PD converts glucose-6-phosphate to 6-phosphogluconic acid and the reducing equivalent are transferred from NADP to NADPH. NADPH is nonenzymatically oxidized by phenazinemethosulphate which transfers the electrons to colourless nitrobluetetrazolium to yield insoluble blue pharmazan derivatives (Grunbaum 1981). The intensity of the blue colour is marked 0, +, ++, +++. The scoring was compared in a double blind manner with the values obtained on quantitative estimation of enzyme activity. The correlation of scoring was excellent. Table 11 depicts data on G6PD deficiency in 2,980 subjects. The normal values range from 8 to 13 IU, whereas values between 0-6 are classified as deficient. As mentioned earlier, in an X-linked autosomal disorder, the female is the carrier and the male children are affected. The data in table 11 indicates that there are as many female deficients as the male children-a paradoxical finding that needs to be explained. Many of the studies carried out by earlier investigators involved sample sizes ranging

from 50-100 and in Jamnagar study it was a tribal population (Aluwalia 1984, Anand et al. 1981 and Pandya 1972). In a recent study in Saudi Arabia (Hazmi & Warsy 1986) 4000 individuals were examined for G6PD deficiency and a sex ratio of 1:1 was observed. Several explanations can be offered for this observation. The easy one would be to say that somehow the process of inactivation of X-chromosome is interfered. But this explanation dose not provide a satisfactory molecular basis for the phenomenon. We had, however, preferred a hypothesis that an autosomal gene product was regulating the expression of G6PD gene located on the X-chromosome. A few months ago Yoshida and his associates (Hitoshi et al. 1989) showed that an N-terminal segment of the enzyme was coded by a gene sequence present on an autosome and the rest of the enzyme by the X-chromosome. It there is a mutation on the autosome one could explain the sex ratio of 1:1. For reasons as yet not clear either due to inbreeding or due to a Founder effect this mutation could have been concentrated in Saudi Arabia and India as historically there is connection between these two populations. At the present time this is a highly speculative concept drawing support from unconfirmed observations (Hitoshi et al. 1989). Alternative hypothesis cannot be ruled out.

Another genetic disorder we examined in a subgroup of the population in order to probe into the effects of inbreeding was pseudocholinestrase deficiency. It was reported that many Vysyas suffer from scolineapoena, i.e. they do not recover rapidly from succinylcholine administered prior to anesthesia. They need to be hyperventilated after surgery and be attached to support systems for very prolonged periods of time.

The enzyme activity was estimated as described earlier (Rao & Gopalan 1979). The enzyme is characterized by a change in the dibucaine and fluoride inhibition numbers. While the normal enzymes has fluoride and dibucaine numbers of 60 and 80, these values are changed for the mutant enzyme (Whittaker 1977).

The data on the activity levels in control in a small group of vysays is shown in figyre 2. It is immediately apparent that the mean value in the Vysyas subjects is almost 50% of that of normals. In many of the individuals the activity is indeed very low. The dibucaine and fluoride numbers are not very different from the control values. These results would suggest that in Vysyas population we have a different type of mutation than is commonly encountered in the Western world. The mutation that was probably present could be of the silent type (Whittaker 1977). The most surprising aspect of this study was the observation that the Vysyas do not practice consanguinity. Infact in the study group there was only one consanguineously related off spring compared to about 33% in the general population (Bittles et al. 1987). Inspite of the absence of inbreeding this endogamous subgroup in an inbreeding mileu has concentrated at a higher level an autosomal recessive mutant gene. The simplest explanation could be that the founders of this group might have had the mutant gene which they could have passed on to the offsprings and the mutant allele could have reached equilibrium.

The studies that I have presented lead to the following conclusions. One is, that considering the population as whole, inbreeding has *not* resulted in a large increase in the incidence of autosomal recessive disorders or marked decrease in fertility or increased infantile mortality. However, in preselected groups such as sick children or among the mentally retarded individuals, an effect of inbreeding is seen. It can therefore be concluded that rather than disturbing the established social practice of consanguinity, it would be advisable to provide genetic counselling to those families that have an affected child.

Acknowledgements

This work was carried out in collaboration with Dr. A.H. Bittles, King's College, University of London, London, UK; Dr. A.N. Radhakrishnan, A. Radha Rama Devi, H.S. Savithri, R.

Ramadevi, J.K. Acharya at the Indian Institute of Science, and Dr. S. Vankat Rao, at Central Food Technological Research Institute, Mysore. Mrs. Seetha S. Murthy, Shantha Kumari, T.N. Veena, and Padmamma in addition to providing able technical assistance charmed more than 100,000 mothers to permit the collection of three drops of blood from the newborn infants, as well answering several questions. Financial assistance from the Indian Instistute of Science, Overseas Development Agency, and British Council Division of the UK Government, and the Indian Council of Medical Research is gratefully acknowledged.

References

Ahluwalia A J 1984 Punjab Survey; *Res. Bull Punjab Univ. Sciences* **34** 153-160

Anand J S, Baxi A J, Nirmal R J, Adalja M R and Anand A K 1981 A study of Glucose-6-Phosphate Dehydrogenase activity in Bhanushali and Non-Bhanushali children; *Indian Paed.* **18** 385-388

Appaji Rao N, Radha Rama Devi A, Savithri H S, Venkat Rao S and Bittles A H 1988 Neonatal screening for aminoacidaemias in Karnataka, South India; *Clin. Genet.* **34** 60-63

Bittles A H and Makov E 1985 Linear regressions in the calculations of lethal gene equivalents in man; *Ann. Hum. Biol.* **12** 287-289

———, Radha Rama Devi A, Savithri H S, Rajeshwari Sridhar and Appaji Rao N 1985 Inbreeding and postnatal mortality in Karnataka, South India; *J. Genet.* **64** 135-142

———, ———, ———, ———, ——— and ——— 1987 Consanguineous marriage and postnatal mortality in Karnataka, South India; *Man. (N.S)* **22** 736-745

Cavalli-Sforza L L and Bodmer W F 1971 (Sanfrancisco: Freeman W.H. & Co.) 341-476.

Darwin G 1875 Marriages between first cousins in England and their effects; *J. Stat. Society* **38** 153-184

Freire-Maia N 1968 *Eugen. A.* **15** 22

Garrod A E 1902 The incidence of Alkaptonuria: A study in chemical individuality; *Lancet* **2** 1616-1620

Grunbaum W 1981 *Hand Book of Forensic Medicine Individualization of Human Blood and Blood Stains Sartorius GmbH;* p 73

Hazmi M A F and Warsy A S 1986 Glucose-6-Phosphate Dehydrogenase polymorphism in the Saudi population; *Hum. Hered.* **36** 24-30

Hitoshi K, Yin Hueng Y W and Yoshida A 1989 Two structural genes on different chromosomes are required for encoding the major subunit of human red cell Glucose-6-Phosphate Dehydrogenase; *Cell* **58** 595-606

Ireland J T and Read R A 1972 A thin layer chromatography method for use in neonatal screening to detect excess aminoacidaemia; *Ann. Clin. Biochem.* **9** 129-132

Luzzatto L 1985 Glucose-6-Phosphate Dehydrogenase; *Advances in Human Genetics* **15** 217-303

Morton N E, Craw J F and Muller H J 1956 An estimate of the mutational damage in man from data on consanguineous marriages; *Proc. natl. Acad. Sci. (USA)* **42** 855-863

Pandya S V 1972 Erythrocyte G6PD deficiency. Dissertation submitted to Saurashtra University

Radha Rama Devi A, Appaji Rao N and Bittles A H 1981 Consanguinity, fecundity and post natal mortality in Karnatak, South India; *Ann. Hum. Biol.* **8** 469-472

———, ——— and ——— 1987 Inbreeding and the incidence of childhood genetic disorders in Karnataka, South India; *J. Med. Genet.* **24** 362-368

Rao P R and Gopalan K B 1979 High incidence of the silent allele at cholinesterase locus 1 in Vysyas of Andhra Pradesh (S. India); *Hum. Genet.* **52** 139-142

Rao P S S and Inbrarj S G 1979 Trends in human reproductive wastage in relation to n-long term practice of inbreeding: *Ann. Hum. Genet.* **42** 401-413

Sanghvi L D 1966 Inbreeding in India; *Eugen. Quart.* **13** 291-301

Sridhara Rama Rao B S, Subhas M N and Narayanan H S 1977 Metabolic anomalies detected during a systematic biochemical screening of mentally retarded cases; *Indian. J. Med. Res.* **65** 241-247

Levels, trends and differentiasls in fertility 1982 (New Delhi, Ministry of Home Affairs:) Vital Statistics Division Office of the Registrar General, India

Whittaker M 1977 *Estimation of Plasma Cholinesterase Activity and the Use of Inhibitors for the Determination of Phenotypes*