HYPOCATALASEMIA: A NEW GENETIC CARRIER STATE *

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Acatalasemia, a rare congenital abnormality characterized by an apparent lack of the enzyme catalase, was first discovered by Takahara and Miyamoto in 1947 (1, 2). The lack of catalase activity in this disease was initially noted in whole blood, but subsequent studies of the tissues of other organs such as the nasal and oral cavities, the pharynx, bone marrow and liver have revealed a similar absence of activity for this enzyme (3–5). Since the initial description of this disease, 38 cases in 17 families have been found and reported in Japan up to April 1959 (6–17); at this writing no reports of acatalasemia have appeared from any other countries of the world.

Shortly after the discovery of acatalasemia it became apparent that the condition probably resulted from homozygosity for a recessive gene (18). Because of the widespread interest in the biochemical identification of genetic carrier states, we undertook a study of persons presumed to be heterozygous for this gene. A recent preliminary report from this laboratory has shown that while certain members of the families of acatalasemic subjects have normal catalase activity in the peripheral blood, others have low values (hypocatalasemia) (19). It was shown further that individuals who, from their position in the family, should be genetic carriers (heterozygotes) are hypocatalasemic. The purpose of this paper is to review the clinical aspects of this disease and its carrier state, to describe in some detail the biochemical procedures utilized in the estimation of catalase values in hypocatalasemics, and to present certain genetic considerations concerning hypo- and acatalasemia.

METHODS OF STUDY

The study team was divided into a) a field investigation unit composed of two members of the Okayama University group and b) four members of the Atomic Bomb Casualty Commission. The field investigation group was responsible for the arrangement of all contacts with the families under study, for reviewing the genealogical relationship among their members, and for the physical examinations. This group collected all blood specimens and delivered them under refrigeration to the laboratory in Hiroshima within a few hours after obtaining the samples. The families investigated are those in which acatalasemia cases were previously detected, and they have been the subject of earlier reports (1, 6, 15, 16).

The standardization and the establishment of normal blood catalase values were performed on subjects at the medical clinic of the Atomic Bomb Casualty Commission in Hiroshima and Nagasaki.[1] The individuals tested were of both sexes and ranged in age from 11 years to over 70.

Blood, collected in a dry syringe, was immediately heparinized, and unless assay was performed shortly

[1] Although no significant difference was noted between the mean catalase value among those exposed and those not exposed to significant radiation, the former have been excluded from the normal control group.

thereafter, was kept under refrigeration. Specimens collected from various areas in Japan were shipped to the laboratory in iced vacuum containers, since storage at 4° for 3 or 4 days was found to have no significant effect on catalase activity.

Hemoglobin was determined by the cyanomethemoglobin method of Drabkin (20) as modified by Crosby, Munn and Furth (21), using a Coleman Junior spectrophotometer.

The assay of catalase activity, based on methods developed by von Euler and Josephson (22), Bonnichsen, Chance and Theorell (23), and Herbert (24), was performed as follows: an appropriate aliquot of whole blood was diluted to 500 ml with distilled water to make a final hemoglobin concentration of 0.14 g per L and mixed gently. Using a blow-out pipet, 1 ml of the diluted hemoglobin solution was placed *immediately* in each of 5 test tubes containing a 5 ml solution of 0.01 M hydrogen peroxide and 0.01 M phosphate buffer, pH 6.8, prewarmed to 37° C in a constant temperature water bath. After exactly 15 seconds' incubation, timed by a stopwatch, the reaction in one tube was stopped by the immediate addition of approximately 2 ml of 2 N sulfuric acid from a beaker; similarly, acid was added successively to three of the remaining tubes at intervals of 30, 45, and 60 seconds. The fifth tube, to which acid was added *before* the diluted blood sample, was used to determine the initial hydrogen peroxide concentration; the peroxide concentration in each tube was determined by titration with 0.005 N potassium permanganate. Under these conditions the disappearance of peroxide follows first order kinetics, and the velocity constant k_1, which may be used as a measure of catalase activity, is calculated for each time interval from the equation

$$k_1 = \frac{1}{t} \log_{10} \frac{x_0}{x}$$

where x_0 is the initial peroxide concentration expressed as milliliters of permanganate and x is the concentration at time t. In this report, the mean of the four values times 10^3, hereinafter referred to as K_{cat}, is used to express erythrocyte catalase activity. Table I shows the data from a typical determination.

The four K_{cat} values for each determination agree closely and do not increase with increasing reaction times up to 1 minute. Analysis of 25 sets of four such readings (selected randomly) yielded a standard deviation of 0.11 K_{cat} units for a single reading within any set of four, the standard error of the mean of the four readings which is taken as the K_{cat} value for the individual thus being 0.055 K_{cat} units. Other sources of laboratory error are minimized by careful control of the conditions under which the assay is performed. As noted by Maehly and Chance (25), a first order reaction is obtained, provided *a*) a short reaction time is employed, *b*) a low substrate concentration and a low ionic strength buffer are used, and *c*) the assay is carried out immediately after final dilution of the blood sample. Temperature in a range between 0° and 60° C has no significant effect on

TABLE I

Data from a typical catalase activity assay

Time	H_2O_2 remaining	$\log_{10} x_0/x$	K_{cat}
sec	ml $KMnO_4$		
0	9.87	0	
15	8.07	0.0874	5.83
30	6.55	0.1781	5.94
45	5.37	0.2643	5.88
60	4.36	0.3548	5.91
		Mean	5.89

K_{cat} values and 37° was found to be suitable and convenient for the present assay. Under these conditions, catalase activity was shown to be linearly related to enzyme concentration using purified human erythrocyte catalase prepared according to the method of Herbert and Pinsent (26). There is also a linear relationship between catalase activity and hemoglobin concentration in normal individuals, a finding previously reported by Funaki (27) and Miller (28), using different assay methods.

In addition to estimating the error of a single reading at 15, 30, 45, and 60 seconds, the variation between duplicate assays on the same sample was also studied. For 18 such duplicate determinations, the standard deviation of the difference between the means of the four readings for each pair was found to be 0.04. The two sources of laboratory variation for which explicit estimates are provided combine to produce a standard error of 0.07 for a K_{cat} determination on an individual. This estimate of laboratory error may be compared with the value 0.73 calculated as the standard deviation of the distribution of individual K_{cat} values, including *both* laboratory error and individual variation.

In establishing the range of normal values, it was found that neither age nor sex appeared to have any significant influence on the level of blood catalase activity. Daily and weekly fluctuations in normal individuals do not seem remarkable; serial determinations in both males and females showed variations not exceeding 5 per cent.

Clinical considerations. There is no gross difference in appearance between the blood of an acatalasemic and that of a normal individual. However, upon the addition of H_2O_2, in contrast to normal blood which remains bright red and foams exuberantly due to the action of erythrocyte catalase, acatalasemic blood immediately turns a black-brown color as methemoglobin is formed, and no foaming occurs. On standing, the black-brown color gradually fades and about 30 minutes later a flocculent yellow-white precipitate containing denatured protein settles out, leaving a clear colorless supernatant fluid containing propentdyopent, the final breakdown product of hemoglobin destruction by hydrogen peroxide (6).

Clinically, approximately 60 per cent of the people with acatalasemia thus far recognized have developed in childhood a peculiar progressive gangrenous disease within

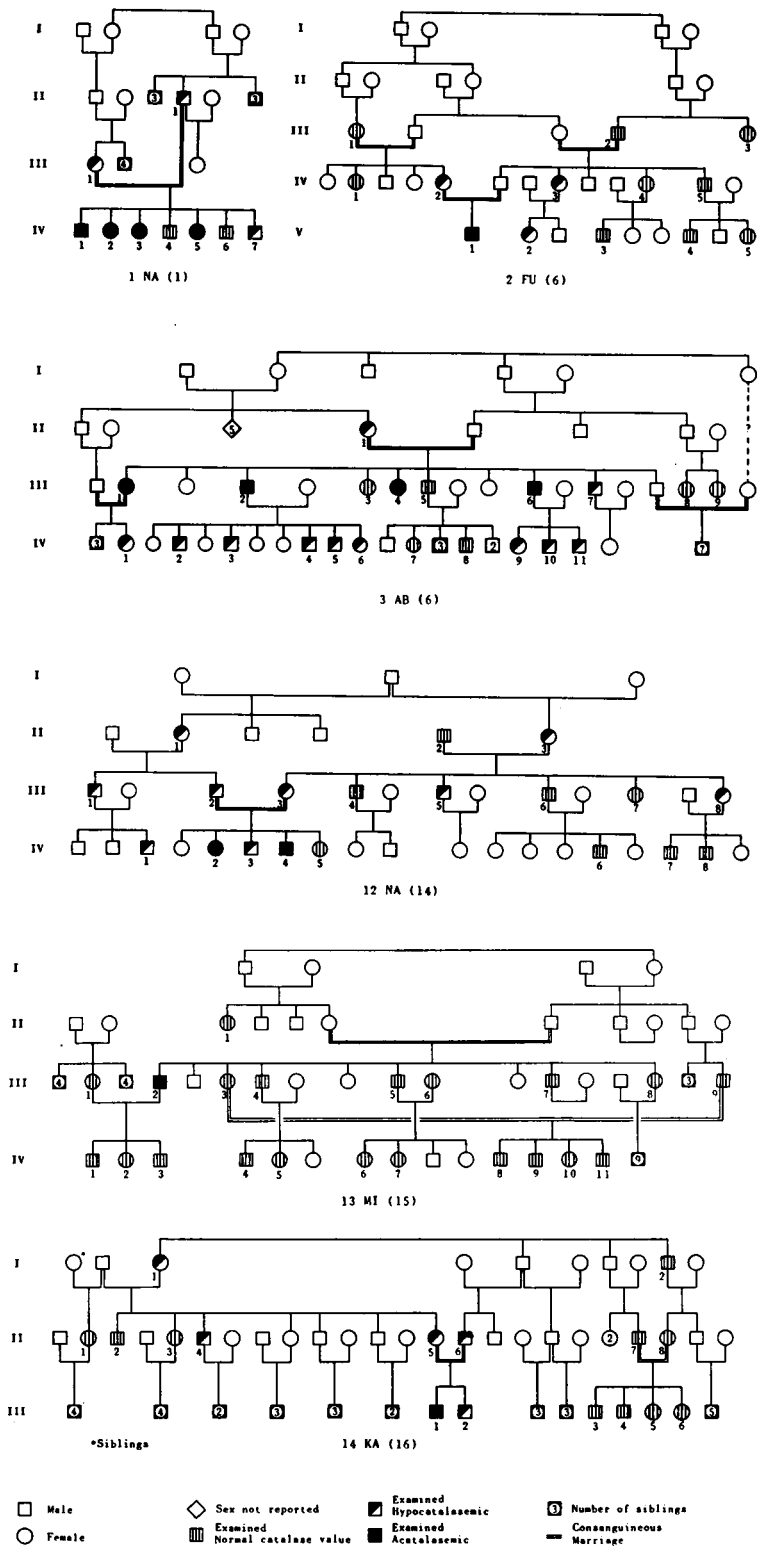FIG. 1. PEDIGREES OF FAMILIES WITH ACATALASEMIA IN WHICH HY-POCATALASEMIA HAS BEEN STUDIED. The families are numbered according to Takahara and associates (29, 30). Each person studied may be located in the pedigree by a Roman numeral indicating generation and an Arabic number indicating position within the generation. Thus, 1 NA IV-2 is the second individual in the fourth generation of family 1 NA.

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the oral cavity (Takahara's disease). In many cases this disease develops at the roots of the teeth but, in some rare instances, the original site may be the crypt of the tonsil. The disease that develops from the roots of the teeth may be classified into three types according to the symptoms: mild, moderate or severe.

In the mild type, repeatedly recurring ulcers with pain are noted in the dental alveoli. In the moderate type, alveolar gangrene develops, and atrophy and recession of the alveolar bone expose much of the neck of the teeth, which loosen and fall out spontaneously. In the severe type, the gangrene of bone or of the soft tissues around the maxilla, mandible or teeth is progressive and presents a picture of widespread destruction. Of the three types, the moderate is the most common (about 50 per cent) while the mild and severe types are seen with about equal frequency.

Takahara (6) believes that the pathogenesis of the oral ulceration may be related to the fact that various bacteria (hemolytic streptococci among them) normally present in the gingival mucosa, may under favorable circumstances rapidly proliferate so that excessive amounts of H_2O_2 are produced. Normal individuals have the capacity to destroy this product, but persons with acatalasemia cannot, and a vicious circle is established. H_2O_2 oxidizes the hemoglobin of the red blood cells, thereby depriving the tissues of the oxygen normally carried by the erythrocytes; the hydrogen peroxide and bacteria invade the surrounding tissues, general tissue breakdown occurs, and gangrene supervenes. Treatment consists of extraction of the diseased teeth or tonsil and the use of antibiotics to control the harmful effects of the offending bacteria. There appears to be no difference in the speed of wound healing in comparison with normal individuals, once treatment has been instituted.

As noted above, about 25 per cent of the acatalasemic patients are asymptomatic, and never develop any form of oral disease. Further, even those who have had some form of disease of the oral cavity in their childhood are in other respects no different from normal people, and apparently after puberty even those individuals who had the severe type of oral disease become symptom-free. Among the 12 cases under observation by Takahara for 10 years or more, aside from showing various manifestations of oral disease in childhood as described above, none has developed any particularly serious disease and all are alive and well at this time.

Description of families studied. Thus far, six-kindreds have been investigated in detail. These are depicted in Figure 1. The numbering of the families corresponds to that employed by Takahara, Doi and Ogura in other reports devoted to a tabulation of all acatalasemics known to date (29, 30). In four kindred (1 NA, 2 FU, 3 AB, and 13 MI), the propositus (IV-2, V-1, III-2, and III-2, respectively) was an individual with the typical signs and symptoms of acatalasemia as described in the preceding section. In the remaining two kindreds, acatalasemia was asymptomatic. In kindred 12 NA, acatalasemia was discovered in the propositus, IV-2,

when H_2O_2 was used in a surgical procedure not involving the oral cavity. In kindred 14 KA, the propositus, III-1, was suspected of having the condition when H_2O_2 was employed in the treatment of pustular eruptions, a suspicion later confirmed by appropriate studies.

RESULTS OF LABORATORY STUDIES

As noted under "Methods," normal standards were established from studies on 259 individuals undergoing evaluation in the Medical Clinic of the Atomic Bomb Casualty Commission (ABCC). The distribution of K_{cat} values among these controls is well approximated by a normal probability curve of mean 5.38 and standard deviation 0.73. The 95 per cent confidence interval of the means is 5.29 to 5.47. The range of control values is from 3.90 to 7.47. One would expect only 2.5 per cent of normal values to fall below the mean minus 2 SD (3.92 K_{cat} units) and only 0.15 per cent below the mean minus 3 SD (3.19 units).

The actual K_{cat} values for the 99 members of the six acatalasemic families studied in detail are shown in Table II; the column "pedigree position" refers to the detailed pedigrees in Figure 1. When the 99 values are arrayed in order of magnitude, 55 are found to lie well within the range (3.90 to 7.47) of ABCC clinic controls and within the range of the observed control mean ± 2 SD (3.92 to 6.84); the lowest of the 55 values is 3.96. The next value in the array is 2.87, which lies more than 3 SD below the observed control mean. There are 31 values in the range 2.87 to 1.48. All other individuals are acatalasemic, with zero K_{cat} values. Thus it appears that there are at least three different groups of individuals within the acatalasemic families: the essentially normal, the hypocatalasemic, and the acatalasemic. The means, ranges, and standard deviations for the K_{cat} values of these three groups, as well as for the controls, are shown in Table III. The hypocatalasemic values are definitely outside the normal range. However, when the 55 values *within* the normal range are compared with the 259 ABCC clinic controls, it is found that the means of 4.97 and 5.38 do differ significantly (p < 0.01). The discrepancy is thus not readily attributable to chance or to sampling variation. On the average the analysis of the blood specimens from the families with acatalasemia was delayed some 24 hours longer than the ABCC control specimens;

TABLE II

Data for families studied in detail

Family	Pedigree position	K_{cat}	Family	Pedigree position	K_{cat}	Family	Pedigree position	K_{cat}	Family	Pedigree position	K_{cat}	Family	Pedigree position	K_{cat}
	II-1	2.62		II-1	2.87		II-1	2.08		II-1	5.05		I-1	1.49
	III-1	1.89		III-1	0.0		II-2	4.05		III-1	6.47		I-2	4.11
	IV-1	0.0		III-2	0.0		II-3	2.16		III-2	0.0		II-1	5.41
	IV-2	0.0		III-3	5.40		III-1	2.25		III-3	5.77		II-2	4.99
1 NA	IV-3	0.0		III-4	0.0		III-2	2.55		III-4	4.03		II-3	5.39
	IV-4	4.41		III-5	6.20		III-3	2.19		III-5	4.87		II-4	2.55
	IV-5	0.0		III-6	0.0		III-4	5.10		III-6	5.64		II-5	1.50
	IV-6	4.77		III-7	2.25		III-5	2.31		III-7	4.86		II-6	2.00
	IV-7	2.21		III-8	5.54		III-6	4.13		III-8	4.04	14 KA	II-7	4.72
				III-9	5.02	12 NA	III-7	5.83		III-9	5.04		II-8	4.89
	III-1	4.74	3 AB	IV-1	2.31		III-8	2.33	13 MI	IV-1	5.03		III-1	0.0
	III-2	4.44		IV-2	2.10		IV-1	1.55		IV-2	4.20		III-2	1.48
	III-3	4.78		IV-3	2.13		IV-2	0.0		IV-3	5.63		III-3	5.95
	IV-1	4.69		IV-4	2.30		IV-3	1.57		IV-4	4.82		III-4	4.88
	IV-2	1.98		IV-5	2.23		IV-4	0.0		IV-5	3.96		III-5	4.89
	IV-3	2.12		IV-6	2.41		IV-5	4.83		IV-6	4.49		III-6	4.52
2 FU	IV-4	4.74		IV-7	5.40		IV-6	4.21		IV-7	4.41			
	IV-5	4.73		IV-8	5.31		IV-7	5.56		IV-8	4.05			
	V-1	0.0		IV-9	2.32		IV-8	6.07		IV-9	6.19			
	V-2	2.45		IV-10	2.28						IV-10	5.49			
	V-3	4.71		IV-11	2.40						IV-11	4.79			
	V-4	5.42												
	V-5	5.07												

the possibility that a slight loss of enzyme activity with age, not detected on the standardization studies, contributes to the observed difference must be considered.

Genetic considerations. The "recessive" inheritance of acatalasemia, first postulated by Takahara, Sato, Doi and Mihara (18), has been amply confirmed by all subsequent family studies. In the 17 segregating sibships thus far reported, the ratio of affected to normal is 36 to 57, with males and females affected in equal numbers. This corresponds perfectly to expectation for a recessive trait, on the assumption of ascertainment through a single affected individual for each segregating sibship—an assumption which, while not strictly

TABLE III

K_{cat} values in controls and in acatalasemic families

Source and type of patients	Number of individuals	K_{cat} Values		
		Mean	SD	Range
ABCC* clinic controls	259	5.38	0.73	3.90-7.47
Acatalasemic families:				
"Normals"	55	4.97	0.61	3.96-6.47
Hypocatalasemics	31	2.17	0.35	1.48-2.87
Acatalasemics	13	0		

* Atomic Bomb Casualty Commission.

justified, provides a reasonable working approach to the data.

With respect to hypocatalasemia, a detailed consideration of the pedigrees shown in Figure 1 (ignoring for the moment family 13 MI) brings out a number of genetically significant facts: a) hypocatalasemic values have been found in every instance in which it has been possible to study one or both parents of an acatalasemic individual; b) the children of unrelated parents, one an acatalasemic and the other presumably normal, are hypocatalasemic; c) the siblings of parents of affected individuals are found to be either normal or hypocatalasemic ; d) the sex distribution among the hypocatalasemics is 17 males and 13 females. In three families (2, 12, 14) it has been possible to trace the defect for three generations. All these findings indicate that hypocatalasemia is the heterozygous carrier state for the gene which, when homozygous, is responsible for acatalasemia.

Family 13 MI is anomalous with respect to the observations noted above. In this family there is one acatalasemic; all other members in the kindred who were tested (among whom are the affected individual's children, several of his siblings and their children, including the offspring of a consanguineous marriage between a sister and cousin) were found to have normal blood catalase activity.
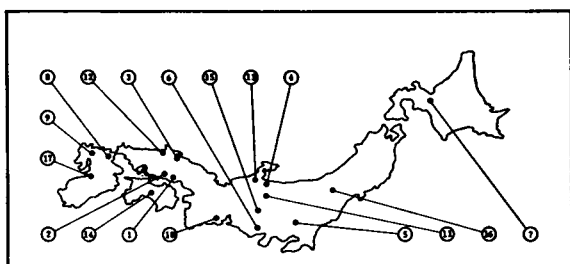
Fig. 2. Distribution of 17 acatalasemic families in Japan.

In this group, the acatalasemic individual and several others were retested six months later with similar results. Under carefully controlled conditions, such an observation would seem best interpreted as indicating a genetic heterogeneity of the acatalasemic phenotype, with the resultant conclusion that there are two (or more) recessively inherited types of acatalasemia, one associated with a definite decrease in catalase activity in heterozygotes, the other not. However, the uncertainties of research in human genetics are such that until satisfactory corroboration of this finding, complete with blood group studies, is available, we shall consider this disease as a single entity.

The clinical significance, if any, of hypocatalasemia remains to be elucidated. There are no obvious ill effects. More specificially and carefully directed questioning has brought out no unusual susceptibility to disease involving the oral cavity. Despite the rarity of (homozygous) acatalasemics, the frequency of (heterozygous) hypocatalasemics is sufficient to make studies on the clinical and subclinical manifestations of this entity both feasible and, in view of the poorly understood functions of this enzyme, desirable. Thus, Figure 2 illustrates the location of the 17 Japanese families in whom acatalasemia has been reported. Neglecting for the moment the earlier discussed possibility that acatalasemia is genetically heterogeneous, a possibility to which we shall return later, then Figure 2 reveals that acatalasemia is not restricted to any one portion of Japan, with the corollary that the responsible gene is one of considerable antiquity which has become well dispersed throughout the country. Under these

circumstances, the frequency of the gene can be *approximated* from the well known relationship

$$q = \frac{c(1 - k)}{16\,k - 15\,c - ck},$$

where q = the frequency of the recessive gene responsible for acatalasemia, k = the proportion of first cousin marriages among the parents of affected persons, and c = the proportion of first cousin marriages among the population as a whole.

In the determination of k, the authors of case reports on material not available to us have been requested to check their earlier observations concerning consanguinity; insofar as rechecks have been possible, the initial reports have been confirmed. Figure 3 summarizes the facts concerning consanguineous parentage in the material of other investigators of this disease. Our own ma-

4 YO (7)   5 KA (7)   6 SA (8)

7 KA (9)   8 YA (10)   9 MO (11)

10 IS (12)   11 MI (13)   15 HA (17)
Remote Consanguinity

16 YO (17)

□ Male
○ Female
◇ Sex not reported
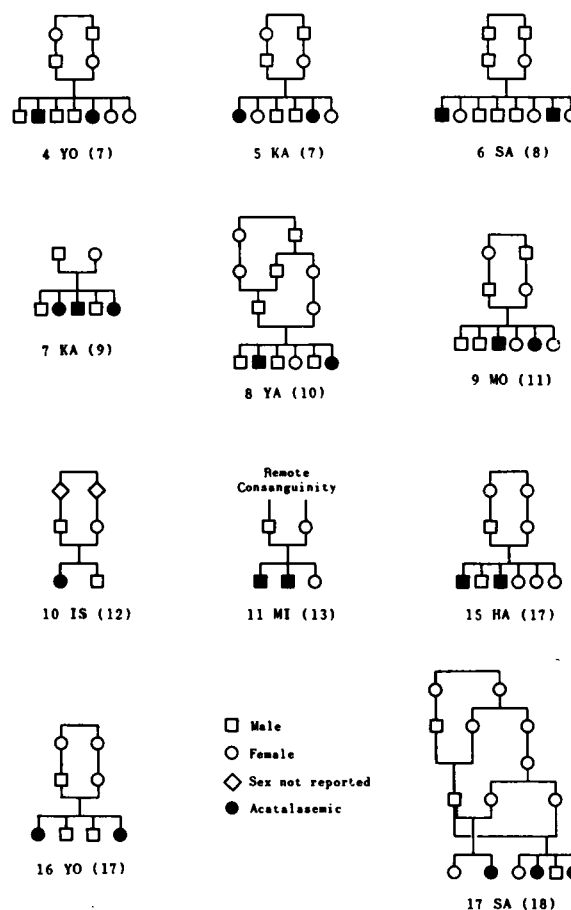● Acatalasemic

17 SA (18)

Fig. 3. Abbreviated pedigrees of families with acatalasemia not studied for hypocatalasemia.

terial has been carefully investigated on this point. From Figures 2 and 3 it can be seen that, for the 17 sibships so far recorded as segregating for acatalasemia, consanguinity of some degree has been recorded in every instance but one. Ten of the 17 marriages involve first cousins, while two involve not only first cousins but additional degrees of relationship. The treatment of these latter in the present context is uncertain, simply because most studies on the frequency of consanguineous marriage fail to record the details in such instances, and control population figures are not available. Disregarding these two marriages, the value of k becomes $10/17 = 0.59$. The value of c is much higher for Japanese populations than for European or American, approximating 0.06 (31). The value of q is thus approximately 0.003. The frequency of heterozygotes is given by the expression $2q (1 - q) (1 - \alpha)$, while the frequency of homozygotes is $\alpha q + (1 - \alpha)q^2$, where α represents the mean coefficient of inbreeding for the individuals comprising the population. In Japan, the value of α approximates 0.004 (31). The frequency of homozygotes with manifest disease should thus approximate 0.000021, while the frequency of heterozygous carriers is 0.005958. In a country such as Japan, with 88,000,000 inhabitants, it would follow from the foregoing calculations that there are approximately 500,000 to 550,000 persons with hypocatalasemia and 1,800 to 1,900 with acatalasemia (assuming a normal life expectancy for both groups). The use of these various formulae is based on the assumption that all acatalasemia is due to a gene change at the same genetic locus, and the further assumption of an essentially uniform distribution of the gene throughout Japan. Since it is the history of genetics that what appeared at first to be discrete clinical and genetic entities have repeatedly been shown to be heterogeneous, and since the distribution of a rare gene in a stable population can only approximate uniformity, these assumptions can only be justified on the grounds that they permit a first estimation of the frequency of these entities that cannot readily be accomplished in any other way. A further assumption underlying the use of these formulae is that of genetic equilibrium in the population. In view of the likelihood that inbreeding levels in Japan have been decreasing

over the past century, here too is a cause to view the foregoing calculations with some reservations. Most of the sources of error in these assumptions and calculations are such as to give inflated estimates of the frequency of hypo- and acatalasemia. However, even if, as is quite possible, the true value of q is of the order of 0.0015, the number of homozygotes still approximates 700, or, with half normal life expectancy, 350, and the number of heterozygotes, 260,000. If, as discussed earlier, there are actually two types of acatalasemia, only one of which is associated with an identifiable carrier state, then the frequency of hypocatalasemia is of course lower. The development of a rapid screening method for the detection of hypocatalasemia would make it possible to check directly on the correctness of the assumptions which have entered into this calculation of carrier frequencies.

Since the discovery of acatalasemia in Japan, there has been a vigorous, continuing search for new cases of the disease. On this basis, there would seem to be a real discrepancy between the number of cases estimated by gene frequency techniques to occur in Japan, and the number actually observed. A clue to this apparent discrepancy may be found in Takahara's clinical observation that some individuals with acatalasemia exhibit little in the way of ill effects. For instance, the discovery of acatalasemia in family 12 NA was quite fortuitous. Thus it is conceivable that in the majority of sibships in which one or more affected individuals occur, the condition is so mild in its manifestations as to be of no clinical significance, and hence it escapes detection.

There remains to be considered the apparently restricted distribution of acatalasemia. Although the discovery of this disease in Japan has sparked an enthusiastic quest for additional cases in that country which far exceeds the effort being expended in other countries, it seems unlikely that this is the entire answer to the lack of case reports from Europe or America. The possibility exists that the gene responsible for acatalasemia will be found to have just as restricted a distribution as genes responsible for hemoglobins C and N in the African Negro (32, 33). One inference from these somewhat analogous situations is that although mutation generally tends to be repetitive in nature, these particular traits owe their occurrence to mutations of great rarity, which by

chance, in some instances aided by selection, have became established in some populations but not in others. Only further research will tell whether acatalasemia is strictly a Japanese gene or whether, like the genetically controlled Diego factor, it will be found in all representative Mongolian populations.

DISCUSSION

In recent years there has been increasing interest in the development of methods for the successful detection of the "carriers" of inherited diseases. By the application of appropriate laboratory tests to relatives of individuals who exhibit some biochemical abnormality as the expression of a hereditary disease, it is possible to detect clinically unapparent variations from normal that are clearly part of an altered genetic pattern. Thus far, laboratory tests, capable of contributing *with regularity* to the detection of the heterozygous carriers of nominally recessive genes, exist for approximately a dozen entities (34–37). However, for only two other diseases, the hereditary hemoglobinopathies and cholinesterase deficiency (38), does there appear to be the precision and ease of diagnosis of the carrier state that exists for acatalasemia. In most other instances, there appears to be substantial overlapping of results between carriers and normals.

The fact that the catalase values of heterozygotes are very nearly intermediate between those of acatalasemics and normal individuals has important implications from the standpoint of biochemical genetics. Although the molecular structure of catalase has not yet been elucidated, it seems reasonable to postulate that acatalasemia involves either an alteration in the amount of enzyme protein produced or an alteration in specificity. From the linearity of the relationship between the amount of active catalase present, and that, therefore, in individuals heterozygous for the acatalasemic gene, the amount of active enzyme protein seems to be very close to half, or slightly less than half, of normal.

The most nearly comparable situation in human genetics thus far explored in detail pertains to inherited variations in the hemoglobin molecule. Hemoglobins A, S, and C, whose production is under control of a series of three allelic genes, differ from one another with respect to only a single amino acid out of the 300 amino acids present in each of the two symmetrical half molecules which comprise human hemoglobin (39, 40). However, in individuals heterozygous for the gene responsible for hemoglobins S or C, only 30 to 40 per cent of the hemoglobin is of the abnormal type (41, 42). Individuals homozygous for either of these genes, with all the hemoglobin abnormal, have a significantly decreased hematopoietic reserve (43, 44). This leads to the conclusion that a probably quite *minor* change in a gene, so small as to result in a single amino acid substitution in a protein, may at the same time very significantly reduce what may roughly be termed "gene efficiency," i.e., as judged by the quantity of end product (45). Otherwise stated, if we envision hemoglobin as a fairly direct reflection of the catalytic activity of a gene or gene-product, then in the case of the hemoglobinopathies it would appear that a change so slight as to involve only a single amino acid in hemoglobin has very significantly impaired the catalytic effectiveness of that gene or gene-product. The same gene change seems to have both qualitative and quantitative results.

The situation in acatalasemia appears to be potentially different. The enzymatic activity of the heterozygote is no more than half of normal—taken at face value, actually 44 per cent of normal, although a large error attaches to this value of 44 per cent. From the results to date it is not clear whether in acatalasemia no protein corresponding to the catalase of the normal individual is being formed, or whether a closely related protein which, however, lacks enzymatic activity is elaborated. It may be possible by techniques currently available to distinguish between these possibilities. If it is the former, then the possibility that the remaining normal allele may be associated, under these circumstances, with less than half the normal quantity of gene product is a puzzling observation, since one must assume that the mutant gene not only results in the absence of the end product normally associated with this locus but also seemingly depresses the activity of its normal allele. If, on the other hand, the alternative of the production of an altered protein which was suggested above is correct, then acatalasemia may be a case in which a mutation is responsible

for a change in the structure of a protein, but it does not alter the "efficiency" of the gene involved, and indeed, if the data are taken at face value, may actually increase the efficiency.

SUMMARY

A clinical, biochemical and genetic study has been made of a rare hereditary disease, acatalasemia, characterized by the absence of activity of the enzyme catalase from the blood and other tissues. Clinically, about half of the acatalasemics suffer from a peculiar type of oral gangrene (Takahara's disease) in childhood. This disease, sporadically distributed throughout Japan, is as yet unreported elsewhere in the world. To date 36 acatalasemics in 17 sibships are known. The high incidence of consanguineous marriage between the parents of the affected persons, the normality of the parents, and the ratio of affected to normal in segregating sibships indicate that the acatalasemic phenotype is recessively inherited.

Heterozygotes, described earlier for this disease, have now been verified, using a biochemical assay for red blood cell catalase activity; the mean activity (K_{cat} in this report) of a normal control group is 5.38 units while that of the heterozygotes is 2.17, and no overlap in values between the two groups has been found. The identification of this carrier state would thus place the gene responsible for acatalasemia in the category of being incompletely recessive (or incompletely dominant). In one of the six families studied, no carrier state could be demonstrated. There are apparently no clinical manifestations in the heterozygotes.

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