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Human Genetics Studies in Areas of High Natural Radiation

I. Methodology[1]

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1. Introduction

Knowledge of the biologic effect of radiation in man has such a large theoretical and practical importance that all efforts must be made to improve it as much as possible. Contrasting to the great importance of the problem, the information directly gathered from man is so limited as to make well-planned investigations even more desirable, especially in the fields of human genetics and public health (the first field is the subject of this paper; for the second one, cf Sax and Gabay, 1960).

A number of papers have been published, especially in the last years, reporting experiments on the genetic effect of radiation in different species of animals and

[1] The preliminary draft of this paper has been presented at the Meeting of Investigators in the Field of Studies in Areas of High Natural Radiation, a meeting sponsored by the World Health Organization (WHO), the Brazilian Academy of Sciences, and the University of Brazil (Rio de Janeiro, December 12-15, 1961).

The " Projeto Espírito Santo ", the main subject of this paper, has been sponsored by the National Nuclear Energy Commission of Brazil, which provided a fellowship to the present author, as well as by the Special Service of Public Health Foundation (Brazil) and the Department of Health of the State of Espírito Santo (Brazil). The field work has been performed under the supervision of the Committee on Human Genetics of the Brazilian Society of Genetics.

plants. Because of their objectivity and accuracy, a number of problems have been satisfactorily solved. Regarding man, some questions still remain unsolved, in spite of the large number of papers published on the subject (Macht and Lawrence, 1955; Crow, 1955; Neel and Schull, 1956; Turpin et al, 1956; Bender, 1957; Tanaka and Okhura, 1958; Schull and Neel, 1958; Kitabatake, 1960; Freire-Maia et al, 1960; Böök, 1962; as well as UN Scientific Committee on the Effects of Atomic Radiation, 1958; The Biological Effects of Atomic Radiation, 1960; Newcombe, 1962, etc).

A few areas are known in the world, especially in Brazil (Fig. 1) and India, where relatively high levels of natural radiation occur (Roser and Cullen, 1962a, b; Effect of

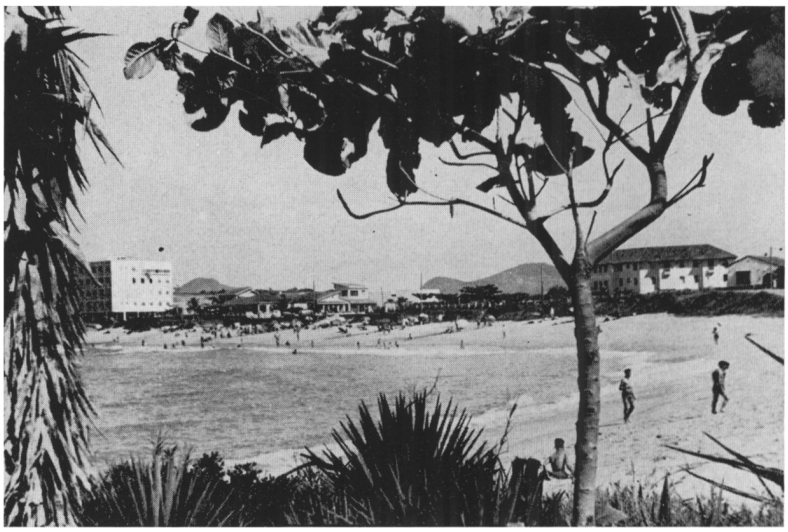

Fig. 1. Several of the Brazilian areas with high levels of natural radiation are located at the beach. The most famous and generally the only known Brazilian beach with high levels of natural radiation is Guarapari, in the State of Espírito Santo.

Radiation on Human Heredity, 1959). Although no conclusive proof has been thus far obtained, the results from experiments made in plants and animals, and the evidences directly gained from man, strongly suggest that natural radiation is at least one of the causes of mutation. It is important to emphasize this problem, especially since some authors, on the assumption that natural radiation is absolutely harmless, have concluded that chestrays in mass « n'offre aucun danger génétique » (Gernez-Rieux et al, 1962). This point of view, supported by some radiologists, has no scientific basis, and is opposite to the opinion of the geneticists who claim that the only level absolutely safe is zero. Of course, the higher the level of natural radiation, the higher will be the rate of induced-mutations. Our limited knowledge on the genetic effects of radiation in human populations, which has been gained more from theoretical inferences than experimental situations (cf e.g. Crow, 1957), raises the

question whether the mutations induced by high natural radiation are or are not presently detectable. There is a great interest in studying the populations living in areas of high natural radiation, but this is not an easy job. On the contrary, it is a tremendous task.

The present paper reviews the investigations in areas of high natural radiation and, although specifically referring to the Brazilian « Projeto Espírito Santo », it also presents and discusses a number of problems inherent to retrospective studies similar to ours (cf Effect of Radiation on Human Heredity, 1959).

2. Investigations in Areas of High Natural Radiation [1]

Several investigations have been or are being performed in order to study the effect of high levels of natural radiation on human beings. In general, the investigators are aware of the possibility of a series of different factors or biases disturbing the results and conclusions. However, we probably all agree that in such an important field it is better to have results subject to some criticisms, and conclusions to be accepted with due cautions, than to have no information.

One of the first studies of the effect of natural radiation on human beings was performed among the uranium miners in Germany (Schneeberg region) and Czechoslovakia (Joachimsthal region). Lorenz (1944), in a critical review of the data obtained, has concluded that about 50% of the miners died of carcinoma of the lung. Radon is believed to be the main, although probably not the only, cause of such a high incidence of lung carcinoma.

Dr. Ross Cameron, county health officer for Washington County, from several years observation of cancer mortality records routinely field in his office, gained the impression that a higher than "normal" incidence of cancer deaths would be occurring in some areas within the county. This was the first step of a project by Dr. Cameron's office and the National Cancer Institute of the Public Health Service. The main goal of the project is to study the correlation between environmental factors and cancer incidence. Preliminary results (Lawrence and Chen, 1959) showed that:

1) The population was extremely stable, but numbered only about 80 000 (so, the probability of studying a large number of cases was relatively low);

2) The computation of rates was made on the basis of census data;

3) Only white individuals entered in the analysis (non-whites are relatively uncommon);

4) The rates were adjusted for age, and data were available by sex and election district;

5) The county did not show anomalous cancer mortality rate. At the time the project was vigorously pressed (1959), it was observed that « the relatively small

[1] In this paper, areas of high levels of natural radiation are just called " radioactive areas " (populations living in those areas are said to be " irradiated populations ").

number of higher background readings seemed to fall in line with election districts showing the elevated cancer rate as calculated by the biometricians " (Lawrence and Chen, 1959). It is evident that such observation must be accepted with due cautions.

Starting from the observation of an " unusually large number of cleft palate patients " in townships of a northern New York county, a project of epidemiological study of congenital malformations was performed in Upstate New York (i. e., N. Y. State excluding N. Y. City) (Gentry et al, 1959). According to data from the N. Y. State Department of Health, some townships showed relatively high rates of congenital malformations. Atomic Energy Commission data showed that such townships presented relatively high levels of natural radiation. Afterwards, other areas with high congenital malformation rates and high levels of natural radiation were found. From these results, an epidemiological study was carried out in Upstate New York. Birth and death certificates were used to get congenital malformation data (no stillbirth data have been used). Certificates of death for children under five years of age (during the period 1948-1955) were matched to their corresponding birth certificates. There was a total of 16 369 cases of congenital malformations, in a total of 1 242 744 live births (giving an incidence of 1.32% congenital malformations). Townships and cities were classified according to their " probable " or " unlikely " presence of relatively high levels of natural radiation. The incidence of congenital malformations in the " probable " and " unlikely " areas were, respectively, of the order of 1.51% (2893/191003) and 1.28% (13476/1051741). The incidence of congenital malformations was found to be higher in the " probable " area for every type of malformation (excluding mongolism, which showed equal incidence). Grouping the countries of the state into six standard geographical regions, the authors found a consistently higher malformation rate in the " probable " towns, when compared to the " unlikely " ones. Differences in father's occupation has revealed not to account for the differences in malformation rates. In some selected townships of high and low rates of malformation, an epidemiological field work was undertaken in order to study eventual differences in the occurrence of known etiological factors. Families were interviewed (around 300 in the " probable " and 300 in the " unlikely " areas), and better radiation data were obtained. The main conclusion was that factors such as medical radiation, rubella, socio-economic status, consanguinity etc, could not account for the differences in malformation rates. In this case, " it would appear desirable to consider radiation as a causal agent " (Gentry et al, 1959).

Data on the incidence of deaths due to congenital malformations in the states within the US and in a number of countries around the world have been taken out by Wesley (1960*a*, 1960*b*) from the Vital Statistics of the US (1958) and the UN Demographic Yearbook (1959). On the assumption that background radiation is entirely produced by cosmic rays, Wesley claims to have found a positive correlation between incidence of congenital malformations and background radiation. Unfortunately, it is not taken in account the possibility of a series of biases and/or different

factors which could account, at least in part, for the differences detected. A rather severe criticism to the paper has been published by Spiers et al (1960), who observe that the paper " ignores relevant experimental observations " and presents a " theory which is not only highly implausible but is incompatible with accepted knowledge ". They observe that " the mechanism for this revolutionary process is not even hinted at ", and are of the opinion that a demonstration " is largely speculative ", the conclusion " has no observational support, « and the calculation (96% of the congenital malformations would be caused by background radiation) " is as fallacious as it is misleading ».

Using US vital statistics data, Kratchman and Grahn (1960) classified the incidences of deaths from congenital malformations in the period 1952-1956 by county and state, and tried to correlate such incidences with geologic environment. In the absence of radiation dosimetry data, they assumed that concentration of radioactivity ore in air, water, rock and soil, are evidence of a higher than average level of natural radiation. The results revealed that the geologic providences with such concentrations of radioactive material may have a higher than average incidence of deaths due to congenital malformations. Although the authors are aware of the possibility of a series of biases, therefore considering their conclusion as " extremely tenuous ", they are of the opinion that the data are " sufficiently provocative to encourage more detailed investigations ".

Using data from baptism records (for analysis of birth sex-ratio and perinatal mortality), death records (for analysis of mortality), marriage records (for analysis of consanguinity), and county (" Municipi ") records (for analysis of causes of deaths), Gianferrari et al (1961) made a preliminary analysis of the data from an investigation in an Italian area with high background radiation (552 mr per year, against 147 mr per year, in the selected control area; i.e., 16.6 r against 4.4 r in a 30-year period, respectively). The authors have estimated an increase in the mutation rates in the radioactive area of the order of 30%-50%. The population size is, however, rather small (1 964 inhabitants in the radioactive area, against 6 304 in the control area, according to data of 1951). The two populations are said bo be similar regarding socio-economic conditions. The incidence of consanguineous marriages is higher in the control area. The analysed data (referring to the period 1838-1958) showed: (1) in a total of 9 151 births in the radioactive area (sex-ratio = 0.5232), and 17 251 in the control area (sex-ratio = 0.5120), no birth sex-ratio difference has been detected; (2) the frequency of precocious mortality (stillbirths plus mortality from birth up to the first month of life) is significantly higher in the control area (0.1202), when compared to the radioactive area (0.1057); (3) the mean age of death is significantly lower in the control area (44 years), when compared to the radioactive one (54 years); (4) in a total of 830 deaths in the radioactive area and 1586 in the control one, during 1940-1958, the frequency of deaths by cancer is significantly higher in the radioactive area (0.1482), when compared to the control one (0.1110). The authors are cautious in their discussions and conclusions, considering the results subject to a number of incertitudes and doubts.

3. The Brazilian « Projeto Espírito Santo »

Although the areas with high levels of natural radiation are of great interest to the students of human genetics, their accurate study is however a matter of great complexity (Stevenson, 1958; WHO, 1959). Accurate results will probably be achieved only through the development of a long-ranged project of prospective human genetic studies, involving the employment of well-trained and specialized teams, and the spending of a large amount of money, during a 10-20 year period.

It was generally held by Brazilian human geneticists (the present author included) that such a prospective project could hardly be undertaken at present in Brazil. It was also our opinion that a retrospective project, although presenting a series of well-known drawbacks and probably leading to inconclusive results (relating to genetic effect of radiation) could well be performed and would be of great value for the planning of an eventual prospective long term project (as a matter of fact, at least presently, no Brazilian human geneticist intends to undertake such a difficult task). Besides the radioactive aspect of the problem, the retrospective project could also bring some interesting contributions in the genetics field of human populations. As a matter of fact, several of these contributions are directly connected to the problem of the genetic effect of radiation in human populations.

As a first approach to the problem in Brazil, a retrospective project was planned and is being carried out (all the data have been collected) (Freire-Maia, 1961). As the great majority of the data would come and did come from the State of Espírito Santo (Fig. 2), the project has been named " Projeto Espírito Santo " (PES). Since the possibility of arriving at positive results (with respect to effect of radiation) is very meager, the main purposes of the project are: (1) to obtain as accurately as possible informations of interest for genetic studies on human populations; (2) to obtain as much information as possible which could be of value for the planning of an eventual long-ranged project of prospective human genetics research in the areas concerned; (3) to obtain information, imperfect as it may be, regarding the predicted genetic effect of radiation in the areas under consideration.

It is very important to emphasize that at no time during the project was any intention sustained to prove (with conclusive results) that natural radiation is inducing genetic effect in the areas investigated. As a matter of fact, the impression was always sustained that such effect probably would not be detected. If some positive result is reached in this matter, however, it will be taken with due cautions and will be considered more as suggestive for further research than as definitive result.

4. Methodology of Reporting

The PES data have been entirely obtained through home visitations by a team of female interviewers (cf Fig. 3), the majority coming from the Department of Health of the State of Espírito Santo. A standard mimeographed questionnaire (cf Appendix) was filled out by the interviewers, the answers being obtained, in the

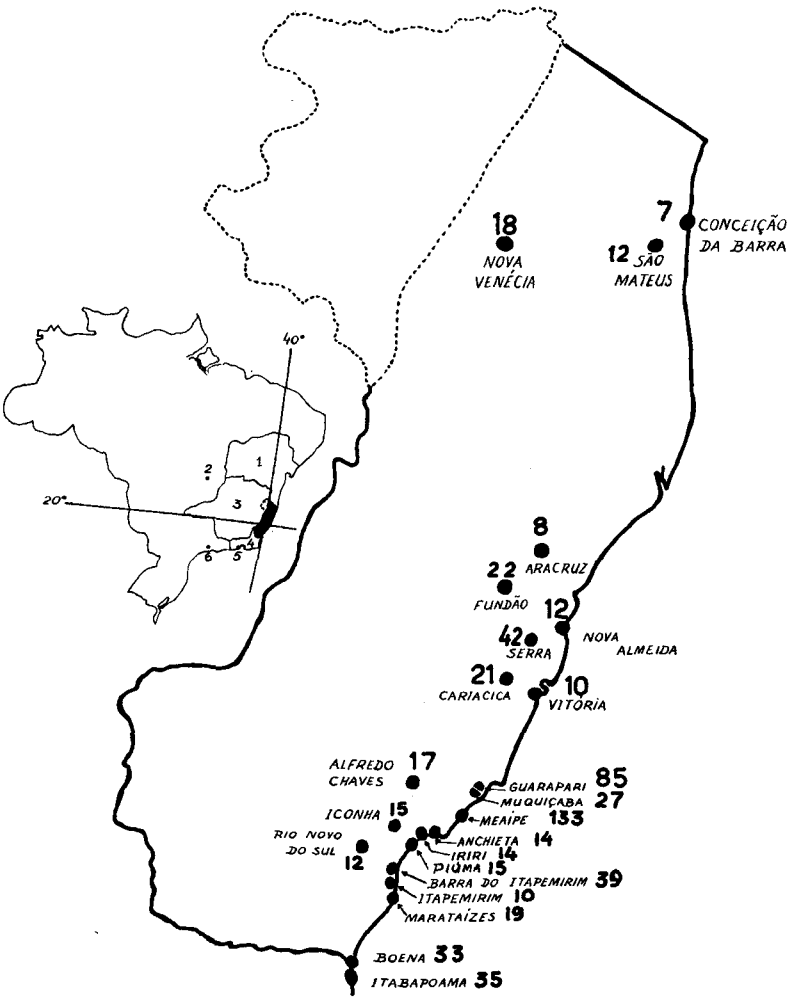

Fig. 2. Map showing the localities studied in the Projeto Espírito Santo, with estimates of their mean levels of natural radiation (in μr per hour). The small map shows: (1) State of Bahia; (2) Brasília; (3) State of Minas Gerais; (4) State of Rio de Janeiro; (5) Rio de Janeiro City, State of Guanabara; (6) São Paulo City.

great majority of the cases, from the wife and/or the husband. Only for some questions (relating to the house, ethnic groups of the spouses and address) the answers were given by the interviewers from their own observations. In special cases, where the informant did not know the answer to a question, or gave supposedly incorrect information, the interviewers wrote their own opinion, stating clearly in the questionnaire that it was their own opinion.

Fig. 3*a*. Due to the absence of conditions of sitting inside the house, one of the interviewers of the Projeto Espírito Santo interviewed outside.

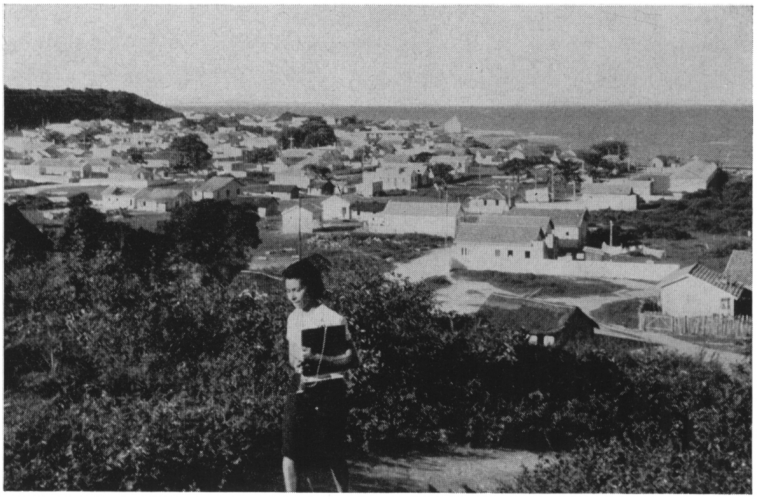

Fig. 3*b*. One of the interviewers of the Projeto Espírito Santo walks up a hill, during the field work, in order to interview some families living there.

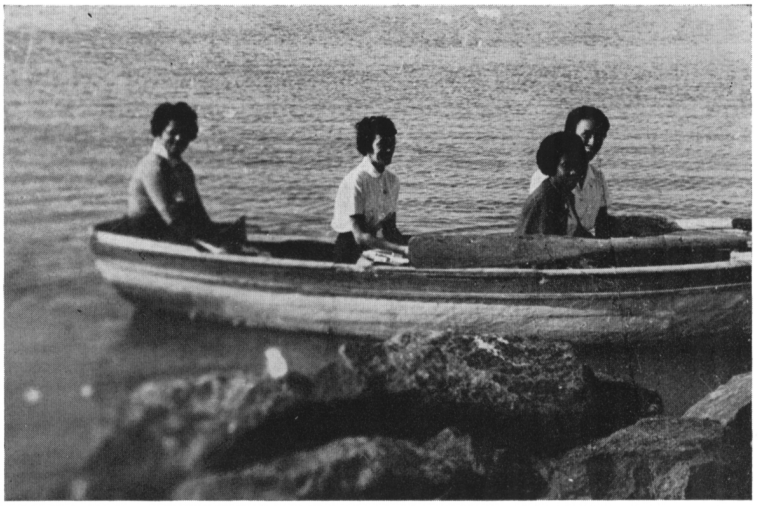

Fig. 3*c*. Some interviewers employed a boat to cross a bay in order to study a small village (Projeto Espírito Santo).

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4.1. Constancy of reporting

Although all efforts have been made to maintain the same interviewing team during the field work, this was only partly possible. The team consisted of three interviewers, from January until the middle of February 1961, when one of them left due to illness. In March, the field work was resumed with the co-operation of five more interviewers, but shortly before the end one more (from the first team) also left. Several reasons (which will be fully discussed in the following pages) made it clear that probably such modifications in the basic team have not altered, in any important way, the collection of the data.

The maintainance of the same team throughout the whole field work is important to avoid variation in the method of reporting. If, however, the interviewers were especially trained to develop as much as possible a standard objective method of reporting, changes in the interviewing team would not be of great practical importance. This is exactly what was done. All the interviewers received a full explanation of the questionnaire's items and on the method of reporting. Under the present author's direct supervision, they were also submitted to theoretical and practical try-outs, both at office and at households in the neighborhood of Vitória, the State Capital. Systematically after each field try-out, there were office meetings at which all filled-out questionnaires were carefully read, mistakes corrected, and new instructions given.

After successive trials during the previous period of field try-outs, a standard questionnaire was developed, with an effort to avoid subjective factors. To exemplify the simplicity and objectivity of the questionnaire, the following is quoted from the written instructions given to the interviewers during the try-outs: " The questionnaire is easy to fill out. Just read the questions and write the answers given by the person interviewed ".

In order to have some idea about the consistency of reporting during the field work, at the end of the collection of the data, a special questionnaire was submitted to the interviewers with express instructions for them to answer the questions individually, carefully and conscientiously. One of the questions read as follows: " During the field work did you always interview people in the same way or did you change your method of asking questions, or the emphasis put in some questions? What changes have you made? " The answers revealed 100% " no change in reporting ".

4.2. The problem of diagnosis

Unfortunately it was not possible to solve satisfactorily the problem of accurate diagnosis. In our preliminary draft of the research plan, as well as in the preliminary report by Frota-Pessoa (1959), the organization of two teams had been suggested. They would work together in the same locality under the present author's direct supervision, with the assistance of an experienced geneticist and a secretary.

The basic teams would consist of two physicians (one pediatrician and one general practitioner) and two social assistants. Eventually other specialists or assistants could also be engaged, as well as at least one social anthropologist (in the case that a sociological research project would not be performed separately).

A number of problems made it necessary to change the preliminary plan outlined by Dr. Frota-Pessoa. It was possible to work with the cooperation of only a few interviewers, and although they were good nurses or social assistants, they did not have the necessary background or training to give accurate descriptions of congenital malformations. However, even if they had had the necessary knowledge, it would have been impossible for them to make an accurate diagnosis of the causes of deaths and abnormalities in absent or dead people, since this drawback is inherent in the retrospective method employed. Although diagnostic errors are probably randomly distributed among the control and irradiated populations, they are inconvenient, as pointed out by the WHO expert committee on radiation (1959); since they " increase the error variance, they magnify the difference which must exist to be statistically demonstrable ". All efforts have been made, however, to ascertain, in the best way, the causes of deaths and detected abnormalities. A large number of abnormalities have been photographically documented in order to make better descriptions afterwards (two of such abnormalities are shown in Fig. 4).

4.3. The " checking "

During the field work, every filled-out questionnaire was immediately checked by the present author (or occasionally by two of the best interviewers) in order to detect and correct mistakes, complete informations, and improve descriptions of abnormalities. A relatively large frequency of filled-out questionnaires presented mistakes and were returned for correction (actually the majority of the mistakes were of minor importance, but considering all important questions we insisted that they be answered correctly and in the best way possible).

Although such checking-up had been done with much care, it would not be completely sufficient to detect all errors and mistakes, both from the interviewer and the informant. In order to get an idea about the magnitude of such undetected errors, a randomly selected sample from the population in each locality was fully re-investigated, as if no field work had been performed previously (checking survey). This re-investigation had a double purpose: (1) to check the information given by the informant, and (2) to check the efficiency of the interviewer. Each informant was warned, in the first visit, that in a few days another interviewer would visit her again and repeat the questions; therefore all the questions should be answered carefully and only reliable information given.

As of yet, the final analysis of the checking survey has not been made, but the comparison of each original, filled-out questionnaire with its corresponding control generally showed differences of little importance. In a retrospective research project, the occurrence of small differences is to be expected.

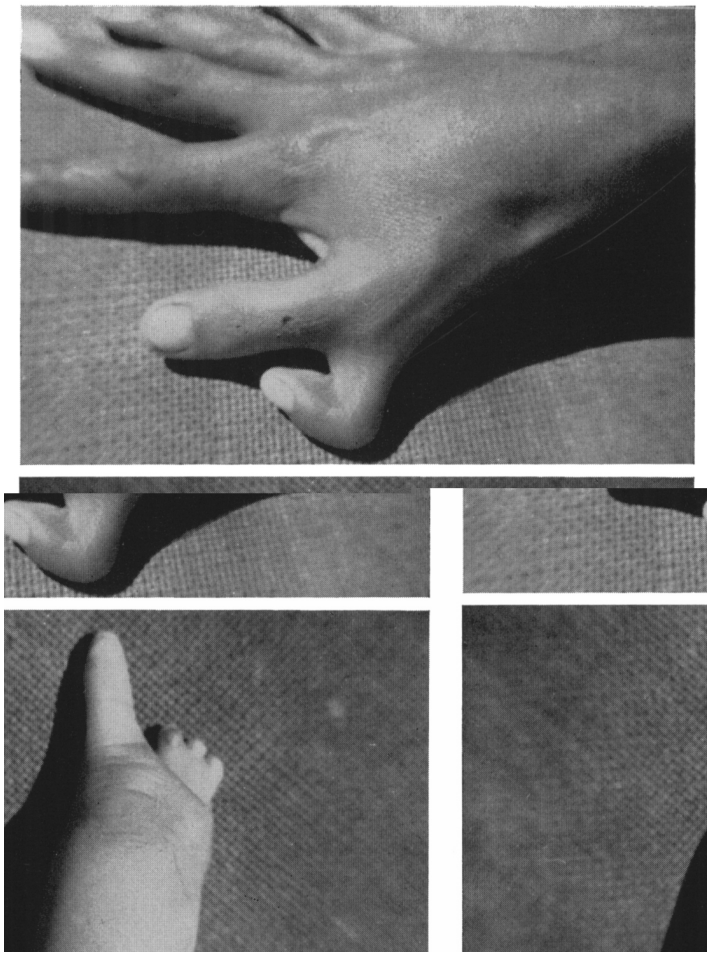

Fig. 4. A number of cases of congenital malformations, found during the field work of the Projeto Espírito Santo, have been photographically documented. Several abnormalities (as the above) have been photographed in detail.

5. Errors and Fallacies

According to the WHO expert committee on radiation (1959), " The types of errors or fallacies which could arise in field studies can arbitrarily be divided into two groups. Firstly, there will be errors which are more or less unique to the area being studied. These could be specified in advance only by someone throughly familiar with the region. Most of these errors will have their roots in an imperfect understanding of the culture of people ".

In an attempt to avoid the first type of error or fallacy, the following steps have been taken: (1) as the present author is not familiar with the region under study, he never entered in direct contact with the people investigated; (2) as the great

majority of the information would probably come from the wives (and did), all the interviewers were females (we believe it easier for women to speak to members of their own sex); (3) the majority of the interviewers had previous experience in contacting people, interviewing and understanding local words and colloquialisms; (4) all the interviewers belong to a low socio-economic class, as the great majority of the people investigated.

The second class of errors is common to all large-scale studies. In this class, the WHO expert committee singled out three sources of errors.

1) *The problem of maintaining uniformity of interview when a number of interviewers are employed.* This problem has been analysed in a preceding section, and the conclusion reached is that, due to constant surveillance and careful indoctrination of the interviewers, the errors introduced were probably negligible.

2) *The problem of keeping the inquirer from knowing to which population the informant belongs.* It is a very important task to avoid differences between the method of reporting data from control and irradiated populations. As long as differences exist, any conclusions will be faults.

From the very beginning of our field work, measures were taken to avoid having the interviewers, the press, people in general and even some authorities indirectly engaged in the project known about our interest in problems related to radiation effect. For all purposes, the study was presented (especially to the interviewers) as intending to analyze exclusively problems related to public health and sanitation (as a matter of fact, this was in part true). In the special questionnaire submitted to the interviewers, one of the questions read as follows: " Why did we choose some localities for study, and not others? Do you think some reason exists for one or more of the localities having been chosen? What are the reasons? What are the localities? ". Only one interviewer believed we had interest in studying the influence of the " ethnic group, foreign ancestrals, or radioactivity " on the frequency of abnormalities, abortions, or stillbirths.[1] She came to this conclusion as she believed " the most important thing in the research project was related to abnormalities, abortions, and stillbirths ", and noted that we studied localities with different incidences of ethnic groups, and localities situated near the coast and in the hinterland. As an example of a locality with high natural radiation, she mentioned only Guarapari, the only one generally known as radioactive among the several studied. When asked in the especial questionnaire about the factors she believed to be responsible for abortions and stillbirths, the interviewer answered that such factors are " organic deficiency, emotional and psychic state, or accident ". She did not mention radioactivity. Concluding the analysis of this section, it can be said that not only the interviewers but even people from the analyzed region did not have any suspicion about our interest in radiation studies. The only interviewer who suspected some interest

[1] All other interviewers said they did not know the answer, or only said that these localities were selected among different areas (North, South, etc) in order to give a better idea of the general situation prevailing in the state.

in radiation studies did not pay much attention to the problem. If, however, some doubt exists about the possibility that she reported differently in Guarapari and the other localities, it is best to mention the special questionnaire where she strongly emphasized (1) that she " always asked the questions in the same way ", and (2) that " during the whole field work I always interviewed people in the same way and did not change anything ".

3) *The problem that intensive observations can hardly be continued for any length of time without the population changing to some degree.* The time elapsed from the inception to the end of the PES field work was not long enough to allow important changes in the populations to occur. The change the WHO expert committee suspected to be the most apt to occur was " an increased awareness on the part of the examined individual of the objectives of the study, and, as a consequence, some loss of perspective ". Such factor probably did not play an important role in our field work since the survey in each locality was made in an intensive rhythm (the collection of the data in each locality generally took no more than a few days, with a mean of 10 days).

Although it is believed that this third factor did not introduce important errors in our survey, an analysis of this point will be possible through the comparative study of the questionnaires filled out at the inception and at the end of the field work in each locality (all questionnaires have been dated).

6. The " Resolving Power " Factors

According to a report by the UN Scientific Committee on the Effects of Atomic Radiation (1958), five points must be considered in order to determine the value or resolving power of a research project intended to study the progeny of irradiated people: (1) the (cumulative) dose to the parents of the individuals under study; (2) the number of individuals whose parents have been so exposed; (3) the number of characteristics of genetic significance to be recorded; (4) the manner in which information on these characteristics is collected; and (5) the availability of a suitable control group.

6.1. The cumulative dose

It is rather difficult to have a reliable estimate of the cumulative dose to the parents of the individuals under study as even in one locality the levels of natural radiation varies enormously from place to place with some closely neighboring spots presenting different levels of radiation. For instance, in Guarapari, State of Espírito Santo, different rates of radiation, in μr/hr, were detected in places no more than a few meters from each other: 40 and 170, 80 and 180, 60 and 200, 60 and 350, etc (Roser and Cullen, 1962*a*).

Of course it would not be completely sufficient to know the mean level of natural radiation prevalent in each locality as people are not fixed, and the places where they are today are not necessarily the same as last week, last year, or 5, 10, or 20

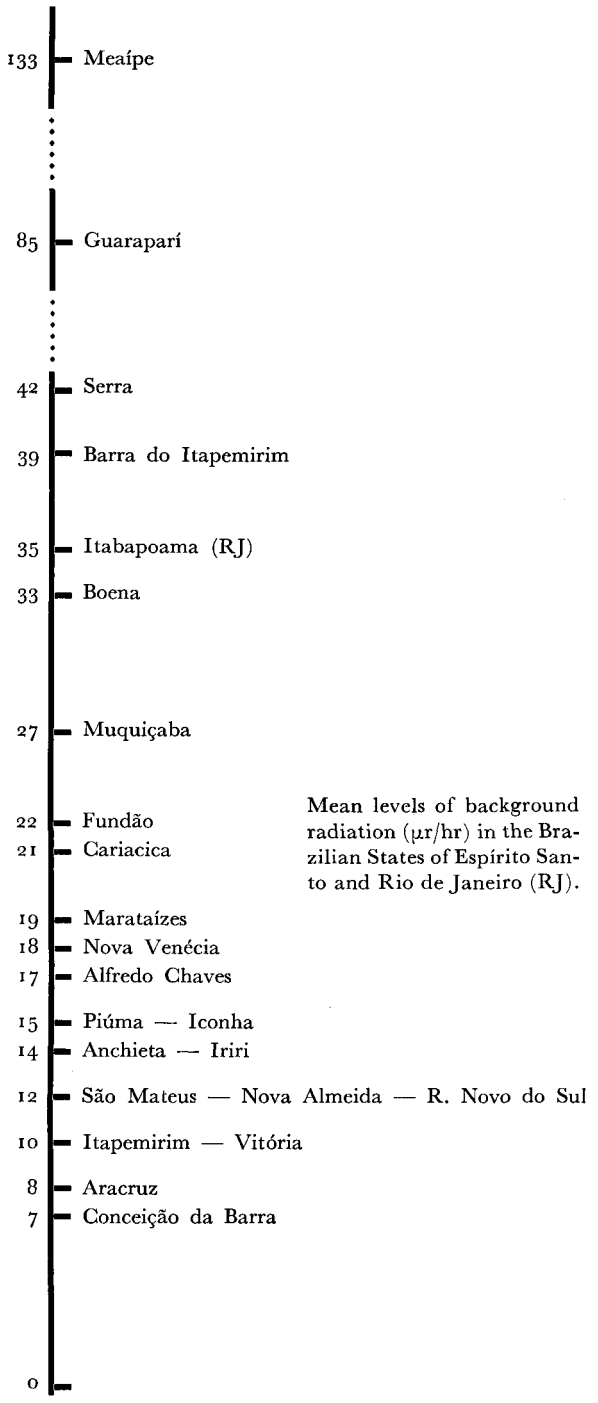

Fig. 5. There is no clear-cut division between areas with high and normal levels of natural radiation in Brazil.

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years ago. If it is difficult to estimate the levels of radiation under which the present population is living, then how can we know the levels for the past generations? It is absolutely necessary however to have estimates of such levels, even if they are not as accurate as we would like. The only present solution is estimates of the mean level of natural radiation prevalent in the localities studied. From Fig. 5, we can see that it is difficult to make a sharp division between areas with high levels of natural radiation and areas with low (normal) ones. A somewhat arbitrary division considers two classes of areas: (1) those with mean levels ranging from 7 to 23 μr/hr, and (2) those with higher mean levels (30–70 μr/hr), or perhaps it would be better to divide the areas into three classes (Tab. I): (1) *Control area* (7–14), (2) *Intermediate area* (16–23), and (3) *Radioactive area* (30–70). Individuals residing in these areas would be submitted to average levels of natural radiation of about 12, 20, and 45 μr/hr respectively (or roughly 0.10, 0.17, and 0.39 r per year respectively), according to the known formula

$$D = \frac{\Sigma_r \, P_r \, X_r}{\Sigma_r \, P_r},$$

where P_r is the population size, and X_r is the mean dose in locality, r (Tab. I). Such levels would be about 3, 5, and 12 r per 30-year period respectively. Assuming a generally accepted 0.63 shielding factor, the respective gonad-doses in a 30-year period would be about 2, 3, and 7 r (this last figure is probably far below the dou-

Tab. I. PES localities, with estimates of their mean levels of natural radiation (X_r) and population sizes (P_r). Estimates have been made on the basis of data by Roser and Cullen (1962*a*, *b*, and personal communication) and by Instituto Brasileiro de Geografia e Estatística (1961)

Control	X_r	P_r	" Intermediate "	X_r	P_r	" Radioactive "	X_r	P_r
Conceição			Alfredo			Nova		
da Barra	7	2229	Chaves	16	1209	Venecia	30	4567
Aracruz	10	1903	Iconha	17	1093	Serra	35	1625
Vitória	10	600[1]	Fundão	20	1510	Itapemirim	40	4095
Nova Almeida	11	1300	Cariacica	23	2339	Itabapoama	40	1800[2]
Rio Novo do Sul	12	2102				Guarapari	70	4260
Piúma	13	1637						
São Mateus	13	6075						
Anchieta	14	1535						
Iriri	14	400[2]						
	ΣP_r = 17781			ΣP_r = 6151			ΣP_r = 16347	

[1] Estimate of the sample analysed, which is a very small fraction of the whole population.

[2] Estimates (Itabapoama is the only locality from the State of Rio de Janeiro, all the remaining localities belonging to the State of Espírito Santo).

bling dose for human genes (Frota-Pessoa and Saldanha, 1960; Freire-Maia, 1960). It is important to note, however, that these are probably minimum values, especially since we did not take into consideration the possibility of internal emitters inhaled or ingested with food or drinking water. These emitters may be more important regarding radiation effect than an external agent (Gentry et al, 1959).

However, the sole analysis of the populations presently living in the control and radioactive areas is not sufficient since a number of people presently living in the radioactive zones came from control areas or are descended from people living or who had lived there (and vice-versa). In order to solve at least part of this problem, we asked people about the localities in which they and their parents had been born and lived. On the basis of such information, each analyzed spouse and his parents can be included in any one of the five categories shown in Tab. II (cf also Tab. III), where class 0 represents the " best control " spouse and class 8 represents " the best radioactive " one (class 9 refers to the cases where information is lacking). Taking into consideration the classification of both spouses (husband and wife), then each couple can be included in one of the 100 classes shown in Tab. III, where class 00 refers to the " best control " couple and class 88 refers to the " best radioactive " one, with a number of intermediate classes (column 9 across and down refer to the cases where information is incomplete).

6.2. The number of individuals

Based on data from the last (1960) Brazilian general census (Instituto Brasileiro de Geografia e Estatística, 1961), we estimated that the control, intermediate, and radioactive localities populations are presently of about 18 000, 6 000, and 16 000, respectively (Tab. I). However, if we consider only two classes of populations, the estimates are about 20 000 individuals living in the control area and 20 000 living in

Tab. II. Values ascribed to the individuals (spouses and spouses' parents), according to their *radiation history*

Radiation history	Spouse	Spouse's Parents	
		(Father)	(Mother)
I. Was born and always [1] lived in HBR [2]	4	2	2
II. Was born, but lived just for a while, in HBR	1-3	1-2	1-2
III. Lived, but was not born, in HBR	0-3	0-1	0-1
IV. Was born and/or lived in *Municipio* whose seat is HBR	0-3		
V. Was not-born and did not live in HBR or in *Municipio* whose seat is HBR	0	0	0

[1] Always is taken as *at least until reproduction.*
[2] HBR = locality with high mean level of background radiation.

Tab. III. Classification of the spouses and couples, on the basis of the values ascribed to the spouses and their parents, according to Tab. I. 00 represents the *best control couple*, and 88 represents the *best irradiated couple*

Husband / Wife	0	1	2	3	4	5	6	7	8	9
0	00	01	02	03	04	05	06	07	08	09
1	10	11	12	13	14	15	16	17	18	19
2	20	21	22	23	24	25	26	27	28	29
3	30	31	32	33	34	35	36	37	38	39
4	40	41	42	43	44	45	46	47	48	49
5	50	51	52	53	54	55	56	57	58	59
6	60	61	62	63	64	65	66	67	68	69
7	70	71	72	73	74	75	76	77	78	79
8	80	81	82	83	84	85	86	87	88	89
9	90	91	92	93	94	95	96	97	98	99

the radioactive one. It is not known how many individuals have been included in our survey (data are presently under analysis), but values of about 7000 couples and 40 000 or more pregnancy terminations seem to be reasonable estimates for the total.

6.3. The number of characteristics

The low level of natural radiation and the small number of individuals, besides other reasons (such as the results by Russell and Russell, 1959, showing that, at least in mice, chronic irradiation is less effective in producing mutations than acute irradiation) makes it unlikely to demonstrate significant excess in the frequency of any genetic abnormality (taken alone) in the radioactive areas. Actually the improbability is still higher due to the fact that we did not have a team of experts to detect, correctly classify the hereditary abnormalities and determine accurately the causes of death. Due to the unfeasibility of studying specific characters assumed to be conditioned by single-gene mutations, our attention was focused on what has been called the population characteristics approach; i.e., the study of genetic characteristics of the population assumed to be influenced by mutations at many loci, as is the case of the sex ratio. With due cautions, other characteristics (birth-rate, death-rate, life expectancy, and fertility), could be and will be used as a basis for evaluating differences between irradiated and control populations. The main information to be taken out from the data are as follows: [1]

[1] (1) by sex; (2) locality and radiation history; (3) ethnic group of the couple; (4) coefficients of inbreeding of the wife and/or the husband; (5) coefficient of inbreeding of the fetus or children; (6) pregnancy termination (abortion, stillbirth, etc); (7) number of co-twins (two, three, etc); (8) ethnic group of the parents of the couple and coefficient of inbreeding of the couple; (9) main region of the body irradiated; (10) country; (11) type of food.

Abortions (1–5), *stillbirths*(1-5), *major and minor congenital abnormalities* (1-5), *children who died in the first year of life (infant mortality)* (1-5), *children who died from the first year of life up to and before getting married* (1-5), *premature children* (1-5), *children born alive* (1-5), *pregnancy terminations* (1-5), *abnormal pregnancy and/or delivery* (1-5), *number of couples* (2-5), *abnormalities of the couple* (1-5), *twins* (1-7), *sibs of the husband* (1-2, 8), *sibs of the wife* (1-2, 8), *conditions of the household* (2-5), *profession of the husband* (2-5), *literacy of the couple* (1-5), *age of marriage* (1-5), *co-habitation time* (2-5), *actual age of the couple and its parents* (1-5), *age of death of the couple and its parents* (1-5), *actual age of the children* (1-5), *age of death of the children* (1-5), *radiographies taken by the couple* (1-5, 9), *radiotherapies and radioscopies made by the couple* (1-5, 9), *nationality of the couple's grandparents* (2-5, 10), *religion of the couple* (1-5), *diet* (2-5, 11).

Special attention will be given to the study of sex ratio. The theory foresees and the experiments corroborate that in the offspring of irradiated females (crossed to non-irradiated males) there is a shift on the sex ratio, diminishing the relative number of males (due to X-linked recessive, lethal mutations induced in the mother's germ-cells); when one irradiates only males, however, the shift is in the opposite direction, diminishing the relative number of females (due to X-linked dominant, lethal mutations induced in the father's germ-cells). However, sex ratio is not as simple a character as one would imagine (cf Novitski and Sandler, 1956; Weir et al, 1958; Szilard, 1960). Although some doubts exist on the reliability of using sex ratio data as indicators of radiation effect in man (Luning, 1961), this ratio seems to be one of "the most likely radiation-determined differences which might be detectable" (Stevenson, 1958). We have gathered data on sex ratio of abortions, stillbirths, and premature, normal and abnormal individuals. Sex ratio data have also been obtained regarding the dead and living sibs of the spouses, their dead and living grand-children (according to the sex of the children), and in some instances even regarding their dead and living great grand-children (according to the sex of the children and of the grand-children).

In the analysis of the data, each detected pregnancy will be classified in one class according to the pregnancy leading to an abortion, stillbirth, infant mortality, etc (Tab. IV). In order to solve the problem of the overlapping categories, specifications will be classed according to the order shown below:

1) Pregnancy termination: abortion or non-abortion;
2) Non-abortion: stillbirth or non-stillbirth;
3) Non-stillbirth: abnormality or non-abnormality;
4) Non-abnormality: infant mortality or non-infant mortality;
5) Non-infant-mortality: post-infant-mortality or non-post-infant-mortality;
6) Non-post-infant-mortality: prematurity or non-prematurity;
7) Non-prematurity: abnormal pregnancy and/or abnormal delivery or "Normal".

The analysis of the population characteristics will not be easy since all of them are probably subject to environmental influences. Efforts will be made, however,

Tab. IV. Each detected conception may be classified as abortion, or stillbirth, or abnormality, etc, as shown according to the pregnancy termination. The possibilities of overlapping categories are also shown (cf text)

From conception up to the 6th month of pregnancy [1, 3]	From 7th up to 9th month of pregnacy [1, 2, 3]	From birth up to the 1st year of life [1, 2, 3]	From the 1st year of life up to the age of reproduction [1, 2, 3]
Abortion	Stillbirth	Infant Mortality	Post-Infant Mortality
Pre-Natal Mortality		Post-Natal Mortality	

to have similar influences in the control and irradiated populations. Regarding this point, some data have been obtained in occordance with suggestions given by the WHO expert committee: (1) geography of the areas occupied by the populations; (2) origin of the groups in question; (3) inbreeding levels; (4) effective population size; (5) migration; (6) diet; (7) cultural and religious practices affecting reproduction; (8) socio-economic conditions; (9) other factors influencing morbidity and mortality patterns.

We will briefly comment on a few of these factors:

Inbreeding levels (3). Depending on the action of selective forces, the possibility exists that in populations long exposed to high levels of radiation, deleterious recessive genes due to increased mutation rates will eventually be disclosed by inbreeding studies (Freire-Maia, 1957). Attempts will be made to compare the load of mutations, defined in terms of lethal and deleterious equivalents per person (Morton et al, 1956), in the control and the irradiated populations. Data have also been obtained on the occurrence of consanguineous marriages among the spouses, their parents, and their married children; this will give us an idea about the inbreeding trends in the present and past generations.

Migration (5). There have been some publications on the migratory currents from other Brazilian states to Espírito Santo. According to the Departamento Estadual de Estatística do Espírito Santo (1948), the number of people, from other states, present in Espírito Santo during the 1940 Brazilian general census was about 110000 (almost 50% came from Minas Gerais; 34% from the State of Rio de Janeiro; around 4% from Bahia, 4% from Maranhao, 2% from Alagoas, and others). (For foreign migration, cf section 5.1).

Cultural and religious practices affecting reproduction (7). It is generally believed that birth control practices are not common in the populations analyzed (data obtained by Dr. Newton E. Morton and co-workers, in a survey of Brazilian Northeastern

[1] Abnormal pregnancy and/or abnormal delivery.
[2] Prematurity.
[3] Abnormality.

populations, revealed that only around 3% of the couples have systematically used, generally during a small lapse of time, an effective contraceptive method (Morton, pers. comm.). Also, there is no suggestion of differential practices in the control and the irradiated populations, and no differing religious influence seems to exist.

Socio-economic conditions (8). Social and economic factors play an important role as causal factors of mortality in general (regarding such influence on stillbirths and neonatal death cf e.g. Baird, 1945). Information on the conditions of the household, profession and literacy of the spouses etc, will be of value to compare the socio-economic conditions prevailing in the control and irradiated populations. Apparently there is no great difference regarding this point.

Other factors influencing morbidity and mortality patterns (9). According to data extracted from the Departamento Estadual de Estatística do Espírito Santo (1958, 1960, 1961b), the following information is representative of the control area (excluding Vitória, the State Capital) and radioactive area, respectively: number of hospitals, 4 and 3; X-rays machines, 0 and 1; delivery or operating rooms, 5 and 2; cradle and newborn beds, 4 and 1; hospital beds, 1 and 3; doctors and pharmacists, 3 and 3; establishments of public health official services, 6 and 6; official doctors, 5 and 5; establishments of primary teaching, 167 and 191. These data, which are to be accepted with some cautions, do not show great differences among the control and radioactive areas.

6.4. The collection of information

It was planned, in the primary outline of the project, to study not only related individuals living in the same house, but also unrelated individuals. As this would cause some extra work in the survey, and bring few benefits since the basic unit in human genetics is the family, the study has been limited exclusively to family data. For this reason and also to avoid duplication of information, efforts were made to find closely related individuals living in different homes.

The problem of collecting information has been treated in a preceding section, (cf Fig. 3), but it is worth repeating firstly, that all the data were obtained by female interviewers through home visitations, and secondly, practically all the residences in the localities studied have been visited, including those located in places of difficult access (Figs. 3*b* and *c*).

There has been no publicity before the arrival or during the stay of the field-work team in each locality (as a matter of fact, even the interviewers generally were told about the next locality to be investigated only a few days before traveling there). Although well-organized publicity could have been useful to enlist the maximum support of the population, it would increase the probability that people being interviewed and the field-work team would become aware of our interest in radiation studies. The increased general-awareness regarding the objectives of the study would give rise to a loss of perspective, as has been observed by WHO expert committee. We did, however, obtain support from the various mayors by telling them that the

research project was sponsored by official health services and that it intended to study the conditions of health and sanitation prevailing in the locality. Consequently permission was granted to carry out the field work. It is not known whether we received maximum support or not, but there is no doubt that the support receivep was very good. For this, we are thankful to the natural receptibility and understanding of the people, to the well-trained and highly-devoted interviewers, and to our sponsoring agencies, Fundação Serviço Especial de Saúde Pública and Departamento Estadual de Saúde do Espírito Santo (no reference has been made, by obvious reasons, to our main sponsoring agency, the National Nuclear Energy Commission).

6.5. The control populations

Control populations, ideal for the PES, are those showing no difference from the irradiated populations, regarding such variables as socio-economic levels, medical assistance, ethnic back-ground, etc. They should differ exclusively in their mean levels of natural radiation. The discovery of such ideal control populations probably could be achieved only through the development of a laborious, long and expensive field work. Due to the impracticability of this, and the impossibility of determining a priori populations which could be considered good controls, and taking also into consideration that, despite the radioactive aspect of the PES, data obtained in any population are of some value for human genetics, no serious attempt was previously made to discover ideal control populations. Efforts were made, however, to use control populations (1) living under known low (normal) levels of natural radiation; (2) living as near as possible to the irradiated populations; (3) showing, at least apparently, socio-economic levels similar to those prevalent in the irradiated populations; and (4) being of size roughly equal to those of the irradiated populations. Of course it is somewhat difficult to demonstrate whether the control populations we have studied are or are not ideal control populations. We expect to have some idea about this problem by analyzing the data on the household conditions, type of diet, ethnic background, profession, literacy, age of marriage, mean reproductive age, consanguinity, and religion. It is possible that at least some of the populations will not be good controls, but in this event they will not be included in the comparative analysis with the irradiated populations.

7. Concluding Remarks

The study of human populations living in areas of high, natural radiation is a very arduous task, presenting problems of difficult solution. It is not known, however, which of these problems can be solved, at least satisfactorily, in a retrospective project. The *Projeto Espírito Santo* (*PES*), besides intending to study the genetic structure of several Brazilian populations (regardless of irradiation) is also an attempt to answer the above question. The project has been planned regarding "the improbability to demonstrate significant genetic changes", and "the desirability of ob-

taining meaningful data, imperfect though they may be " (WHO, 1959). Although the probability of achieving a positive result is insignificant, " our very lack of knowledge as to what to expect means that there might be a surprise " (cf Stevenson, 1958). However, no conclusion can be advanced, since the data are still under analysis.

Summary

This paper reviews the investigations in the field of the effect of natural radiations on human populations, and discusses a number of problems inherent to retrospective genetic studies of human populations living under high levels of natural radiation. The solutions to face, at least partly, such problems are presented and discussed with special reference to the Brazilian " Projeto Espírito Santo " (PES).

In summary, the main PES subjects presented and discussed are as follows:

1) The data, including about 7 000 couples and 40 000 pregnancy terminations, have been fully collected and are presently under analysis. They were gathered from January to October 1961 by a team of specially-trained female interviewers employing a standard questionnaire, making home visitations, and being directly supervised by the present author;

2) The reporting has been practically consistent both within and between populations throughout the whole field work. Biased contribution both from the interviewers and the informants was probably negligible. The collection of the data was not subject to any subjective factor related to problems of radiation effect.

3) The interviewers came from the same socio-economic level from which the majority of the people interviewed belong. They were rather familiar with the region and had a perfect understanding of the culture of the people.

4) A *checking survey* was performed to estimate the reliability of the data, with good results.

5) No propaganda or publicity has been made preceding or during the field work. This reduced the probability that people and the field work team knew o our interest in radiation problems.

6) Although efforts were made to have good descriptions of the abnormalities, and good information on the causes of deaths, unfortunately accurate diagnosis was not possible.

7) Cultural and religious practices probably do not affect differently reproduction in the radioactive and control areas, and socio-economic conditions seem to be roughly similar.

8) It is difficult to know the cumulative radiation dose to the parents of the individuals analyzed (an attempt regarding this point will be made when the data are analyzed). The mean individuals living in the control, intermediate and radioactive areas are probably subject to minimal gonad-doses of about 2, 3, and 7 r in a 30-year period, respectively.

9) No attempt was made to study the influence of radiation on the rate of mutation at specific loci, but an analysis will be made of the genetic characteristics of the populations assumed to be conditioned or influenced by mutations at many loci.

10) As has been duly pointed out in this paper, the " Projeto Espírito Santo " (PES) was *not* specifically planned to demonstrate, with conclusive results, the genetic effect of natural radiation. Although some positive result is possible, it is highly improbable.

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RIASSUNTO

In questo lavoro vengono presentate le ricerche effettuate sugli effetti delle radiazioni naturali sulle popolazioni umane, e vengono discussi diversi problemi inerenti a studi genetici retrospettivi su popolazioni umane in condizioni di elevati livelli di radiazioni naturali. Vengono presentate e discusse le soluzioni atte a risolvere, almeno in parte, tali problemi, con particolare riguardo al progetto brasiliano « Espírito Santo » (PES). Riassumendo, i punti *principali del progetto sono i seguenti:*

1. Sono stati raccolti, e sono in corso d'analisi, dati riguardanti circa 7000 coppie e 40 000 gravidanze a termine. La rilevazione fu effettuata, dal gennaio all'ottobre 1961, da un gruppo di intervistatrici qualificate sulla base di un questionario standard, con visite domiciliari, e con la diretta supervisione dell'autore.

2. Nel corso dell'intera ricerca, la rilevazione si è dimostrata uniforme, sia nell'ambito di una stessa popolazione che tra popolazioni diverse, per cui l'errore degli operatori deve essere stato trascurabile. La raccolta dei dati non è stata soggetta a fattori soggettivi relativi ai problemi degli effetti delle radiazioni.

3. Le intervistatrici appartenevano allo stesso livello economico-sociale a cui apparteneva la maggioranza della popolazione intervistata. Esse avevano familiarità con la regione e con le condizioni culturali della popolazione.

4. Accertamenti atti a stimare l'attendibilità dei dati hanno dato buoni risultati.

5. L'assenza di qualsiasi propaganda o pubblicità, prima o durante lo svolgimento del lavoro, ha ridotto la probabilità che venisse conosciuto il nostro interesse sui problemi delle radiazioni.

6. Pur cercando di ottenere buone descrizioni delle anormalità e informazioni precise sulle cause di morte, non è stato possibile effettuare diagnosi precise.

7. Sembrerebbe che le pratiche culturali o religiose non incidano differentemente sulla riproduzione nelle aree radioattive e di controllo, e che le condizioni economico-sociali siano più o meno simili.

8. È difficile conoscere la dose cumulativa di radiazioni nei genitori degli individui esaminati (un tentativo su questo punto sarà fatto quando i dati saranno analizzati). L'individuo medio, vivente nelle aree radioattive, intermedie e di controllo, è probabilmente soggetto a dosi gonadiche minime, rispettivamente di circa 2, 3 e 7 in un periodo di 30 anni.

9. Non è stata studiata l'influenza delle radiazioni sul tasso di mutazione genica, ma sarà svolta un'analisi delle caratteristiche genetiche delle popolazioni presumibilmente condizionate o influenzate da numerose mutazioni.

10. Come già rilevato, il PES non è stato specificamente condotto per dimostrare in maniera conclusiva l'effetto genetico delle radiazioni naturali. Per quanto sia possibile, è tuttavia molto improbabile avere dei risultati positivi.

RÉSUMÉ

Ce travail concerne les recherches sur les effets des rayonnements naturels chez les populations humaines et discute les différents problèmes relatifs aux études génétiques rétrospectifs de populations humaines exposées à des niveaux élevés de rayonnements naturels. Les possibles solutions de ce problème sont présentées et discutées, notamment en ce qui concerne le Projet Brésilien « Espírito Santo » (PES). Les points principaux de ce projet sont les suivants:

1. Les données concernant 7000 couples et 40 000 grossesses à terme ont été recueillies et se trouvent en cours d'analyse. La collection des données fut effectuée, de Janvier à Octobre 1961, par une équipe d'interviewers qualifiées, sur la base d'un questionnaire standard et visites domiciliaires, sous la directe supervision de l'auteur.

2. Au cours de la recherche, la collection des données a été uniforme, au sein de la même population ainsi que chez des populations différentes; l'erreur des opérateurs peut donc être minimisé. La collection des données n'a pas été influencée par des facteurs subjectifs concernant le problème des effets des rayonnements.

3. Les interviewers appartenaient au même niveau socio-économique de la majorité de la population interviewée. Elles connaissaient bien la région et les conditions culturelles de la population.

4. De bons résultats ont été obtenus par l'évaluation de la crédibilité des données.

5. L'absence de toute propagande ou publicité, avant et pendant le développement de la recherche, a réduit la probabilité que notre intérêt sur le problème des rayonnements fût connu.

6. Tout en cherchant d'obtenir de bonnes descriptions des anormalités et de précises informations sur les causes de mort, il n'a pas été possible d'effectuer de diagnostics précis.

7. Il paraît que les pratiques culturelles ou réligieuses n'exercent pas une différente influence sur la réproduction dans les régions de radioactivité et de contrôle, et que les conditions socio-économiques soient plus ou moins les mêmes.

8. Il est difficile de connaître la dose cumulative de rayonnements chez les parents des individus examinés (une tentative sera faite une fois complétée l'analyse des données). L'individu moyen, vivant dans les régions radioactives, intermédiaires et de contrôle, est probablement sujet à des doses gonadiques d'un minimum respectivement de 2, 3 et 7, sur une période de 30 ans.

9. L'influence des rayonnements sur les taux de mutation génique n'a pas été étudiée, mais une analyse sera faite des caractéristiques génétiques des populations vraisemblablement conditionnées ou influencées par de nombreuses mutations.

10. Le PES n'a pas été spécifiquement conduit pour démontrer de façon conclusive l'effet génétique des rayonnements naturels. Même si possible, il est toutefois très improbable d'avoir des résultats positifs.

ZUSAMMENFASSUNG

Die Arbeit beschäftigt sich mit den Untersuchungen über die Wirkung der natürlichen Strahlungen auf die menschlichen Bevölkerungen. Es werden dabei verschiedene Probleme erörtert, die sich aus retrospektiven genetischen Forschungen über die menschlichen Population ergeben, die hochgradigen natürlichen Strahlungen ausgesetzt sind. Es folgen Aufzählung und Erörterung der verschiedenen Möglichkeiten, diese Probleme wenigstens teilweise zu lösen. Diesbezüglich wird besonders auf das brasilianische Projekt « Espírito Santo » (PES) hingewiesen. Zusammengefasst sind die wichtigsten Punkte dieses Projekts folgende:

1. Über ca. 7000 Ehepaare und 40000 ausgetragene Schwangerschaften wurden Erhebungen angestellt, die zur Zeit noch in der Analyse sind. Die Daten wurden in der Zeit von Januar bis Oktober 1961 von einer Gruppe ausgebildeter Interviewerinnen erhoben, welche die Probanden einem Standard-Fragebogen gemäss zu Haus unter direkter Aufsicht des Verf. befragten.

2. Im Laufe der gesammten Untersuchung fielen die Ergebnisse sowohl im Rahmen einundderselben Population als bei verschiedenen Bevölkerungen gleichmässig aus, woraus zu schliessen ist, dass der Fehleranteil seitens der Untersucher minimal gewesen ist. Bei Erhebung der Daten wurden die subjektiven, mit den Problemen der Strahlungen zusammenhängenden Faktoren nicht in Betracht gezogen.

3. Die Interviewerinnen gehörten alle den gleichen sozialwirtschaftlichen Kreisen an, wie die Mehrzahl der befragten Bevölkerung. Die geographischen und kulturellen Verhältnisse derselben waren ihnen jeweils gut bekannt.

4. Ermittlungen, um die Verlässlichkeit der Erhebungen zu beurteilen, tätigten gute Resultate.

5. Es wurde bewusst jegliche Propaganda oder Reklame vor und während der Abwicklung der Untersuchung vermieden, damit unser Interesse für die Probleme der Strahlungen möglichst wenig bekannt wurde.

6. Obwohl versucht wurde, eine gründliche Beschreibung der Anomalien und genuae Angaben über die Todesursachen zu erhalten, so war es doch nicht möglich, exakte Diagnosen zu stellen.

7. Es scheint, dass die religiösen und kulturellen Gebräuche sich sowohl in den radioaktiven als in den Kontrollgegenden in gleichem Masse auf die Fortpflanzung auswirken und dass die wirtschaftlichen und sozialen Verhältnisse mehr oder weniger die gleichen sind.

8. Die akkumulative Dose der Strahlungen bei den Eltern der untersuchten Personen lässt sich sehr schwer erkennen (ein diesbezüglicher Versuch wird unternommen werden, wenn die Daten analysiert sind). Der Durchschnittsmensch der radioaktiven, der intermediären sowie der Kontrollgegenden ist wahrscheinlich im Laufe von 30 Jahren minimalen Gonadendosen von ungefähr 2 bzw. 3 und 7 r ausgesetzt.

9. Der Einfluss der Strahlungen auf den Prozentsatz der Genmutationen wurde nicht untersucht, jedoch werden die Erbmerkmale der Bevölkerung, die vermutlich durch zahlreiche Mutationen bedingt oder beeinflusst wurden, analysiert werden.

10. Wie bereits erwähnt, war es nicht das spezifische Ziel des PES, den Erbeinfluss der natürlichen Strahlungen endgültig zu beweisen. Im Rahmen des Möglichen erscheint es allerdings unwahrscheinlich, positive Resultate zu erlangen.

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APPENDIX A

STATE DEPARTMENT OF HEALTH
SPECIAL SERVICE OF PUBLIC HEALTH

QUESTIONNAIRE

Conditions of Sanitation and Public Health in the State of Espírito Santo

..... Locality ..... Wife ..... Husband ..... Number of the questionnaire

HOUSE: Is the house your own? ( ). Walls . . . (state of repair). Roof . . . . (state of repair). Floor . . . . (state of repair). Do you make use of water from the . . . .( ), river ( ), fountain ( )? Is there a bathroom? ( ) and lavatory sink in the house? ( ). Is there a toilet (USA)? ( ); a WC ( ); a dry cesspool? ( ); connected to the main drains? ( ). Is the rubbish collected by the Prefecture? ( ); is it exposed? ( ); is it burned? ( ); or is it buried? ( ).

WIFE AND HUSBAND: Have you children from another person? Wife ( ), Husband ( ). Is this your first marriage? ( ).

Where did you get married? . . . . . In which year did you get married? ..... How long have you been married? . . . .

Couple	How old were you when you married?	How old are you?	What is your profession?	Do you read? write? Degree of instruction
Wife				
Husband				

Couple	In which year were you born?	Ethnic groups (W, DW, LM, DM, N),[1]			How many siblings have you? [2]
		Own	Father	Mother	
Wife					
Husband					

[1] W = White; DW = Dark White; LM = Light Mulatto; DM = Dark Mulatto; N = Negro
[2] MA = Males Alive; FA = Females Alive; MD = Males Dead; FD = Females Dead

CHILDREN: How many living children have you? . . . . . How many dead? . . . . . How many were born dead? . . . . How many abortions and miscarriages have you had? . . . .

Children who *have been born alive*:

Order	Male (M) or Female (F)	Name	Age	Place of birth	Present residence	Married? Single?[1]	Died	
							Age	Cause
1°								
2°								
3°								
4°								
⋮								

[1] In case of married, widowed, etc, persons, enter on the back the number of children alive (males and females) and the number of children dead (males and females).

	How many?	Between which children?	How old?	Sex	Cause	Defects and anomalies
Abortions plus Miscarriages						
Stillbirths						

How many twins? (Indicate)
Which are the children who were premature? (Indicate order, age, and cause of prematurity)
Which are the children who were born with some defect or anomaly? (Indicate the children and describe the anomalies)
Which ones are or were sick? (the same)
Wife, husband, and family of both: defects, anomalies, sickness, deaf-mutism (congenital)
What were the courses of the pregnancies?
of the deliveries?
What illnesses did you have during the pregnancies? (rubella?) (Indicate ages)

	Radiographies (X-rays)			(1) Radiotherapy (2) Radioscopy			Which is the relationship?		
	N	Regions	Time	N	Regions	Time	Father	Mother	(a) couple (b) children
Wife									
Husband									

Irradiation during pregnancies (type, n. regions, pregnancies):
Spouse died _____ years ago; death age _____; cause of death _____

Where were they born and where did they live? (Indicate ages)

	Spouses	Father	Mother
Wife			
Husband			

Have you any foreign-born grandparents? Wife ( ), Husband ( )
Which ones? (Indicate countries)
What is the wife's name? Religion?
What is the wife's father's name?
What is the husband's name? Religion?
What is the husband's father's name?
Full address
Length of co-habitation

....
Informant (s) Date Inquirer

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APPENDIX B

Discussion at the WHO Meeting of Investigators in the Field of Studies in Areas of Natural Radiation

F. M. Salzano. I would like to comment on several points:

1) Do you believe that the problem of some people not having cooperated with the interviewers will affect the results of the survey?

2) How will you analyze the data from the control and irradiated populations, taking into consideration the problem of the ethnic background?

3) How did you estimate the gonad-dose?

4) Don't you think it would be much less expensive and more practical to gather only consanguinity data and compare the genetic load in the control and irradiated populations?

Ademar Freire-Maia.

1) The number of couples who did not cooperate during the field work could be counted on one hand and are therefore insignificant.

2) The family data will be analyzed separately, according to locality, the radiation history, and the ethnic background of the couples (that is to say, the data regarding white couples from control populations, for instance, will be compared only to data regarding white white couples from irradiated populations, and so on).

3) The data presented in Tab. I refer exclusively to environmental radioactivity. Not all of this however (approx. 63%) will reach the gonads.

4) No, as it leaves the problem of knowing whether the inbred and outbred samples (both in the control and irradiated areas) are or are not comparable samples, regarding socio-economic variables. It is important to know whether or not socio-economic factors play different roles in the control and radioactive areas. It is my impression that good data can be achieved only through a field work similar or better than ours.

Newton Freire-Maia. I think Dr. Salzano's suggestion could be used as the addresses of consanguineous couples can be obtained in the seat of the Diocesis, and/or in the Parish files. Consanguineous couples could then be contacted through home visitations. Of course there is the problem of changing in address with the resultant that a number of consanguineous families would not be ascertained. This presents a serious problem, due to the relatively small number of consanguineous marriages.

C. Pavan. I would just like to make an addition to what Dr. Newton Freire-Maia said. Although Dr. Salzano's suggestion is good, I think that it could not be applied in the populations of Espírito Santo, due to their small size.

H. Jakobi. The excellent work done by Fathers Roser and Cullen shows, and we know that there are quite different levels of radiation in the radioactive areas studied. I can imagine that a person, living only one year in direct contact with the radioactive sands,

will receive much more radiation than another person living 30 years in the same locality but not in direct contact with monazitic sands. I don't know if you have taken this into consideration.

Ademar Freire-Maia. I fully agree with Dr. Jakobi. I know of these differences and they will be taken into account. The number of people subject to large levels of natural radiation is, however, very small, and the figures I have presented are estimates for the general population.

C. Chagas (President of the 1st session). I would like to accentuate an interesting aspect of the work by Fathers Roser and Cullen: in the individual measures they made, the dose received is more or less constant. This makes it possible to estimate the mean dose received by the mean inhabitants of a certain region.

A. C. Stevenson (President of the 2nd session). I am not quite sure what lines you would like our discussion to follow. If I open with a few remarks and try to be provocative, this might start the discussion satisfactorily.

It seems to me that we all entirely agree with Dr. Ademar Freire-Maia, that it is most unlikely that we could detect differences attributable to increased mutation between populations of these sizes which were exposed to these differences in radiation. The study which we are considering is one involving people living in a characterized area and those living in control areas. If this is so, it is basically an epidemiologic study rather than a genetic study. As there are so many gaps in our understanding, even of what to expect, we have to be careful not to get involved in making comparisons of abstractions even if we have in the back of our minds a particular interest in a concept of genetic load.

I do not think it wise to talk about making comparisons of genetic loads in different areas. These are theoretical considerations which can be dealt with later. What you need to do is to make comparisons of differences in the things which characterize these populations. The only reason for having the abstractions or hypotheses is that you wish, in so far as possible, to collect data and information with which you can test the maximum number of hypotheses. For any epidemiologist having several hypotheses this is unsatisfactory. What we should like, is to have as simple a hypothesis as is possible, collect the information only when it is strictly relevant to the needs of our comparison and to try and control those factors which are irrelevant. This is not easy to do in this situation.

Now, the information that we want is of biological variation which is due to inborn factors, minimizing or controlling those factors which we would expect to determine environmental variations. Let us take one example: supposing we wish to compare the situation in two populations in respect to stillbirth rate. This would be perfectly legitimate. Stillbirth frequency is a good social indicator, but it is also a parameter that might have a variation due to inborn factors. So even if we standardized in our comparison say for maternal age and sex, we still could not interpret differences.

I should like to ask Dr. Ademar Freire-Maia to give us more information about these areas. The only indication we have had on the situation of medical services in these areas was the casual remark of Father Roser about the small hospitals. It would be of interest to know the number of doctors or hospitals of any kind. Are there any vital statistical data collected in respect to these areas, any recent census data or any medical data however crude? Have we information on the sex distribution of the population in these areas? If

any such information is available, perhaps it would be the first approach, both to selection of control areas and the first crude comparisons.

In experimental genetics, as far as poor human geneticists can see, no organism ever dies unless they have a lethal gene! We cannot accept this as true, and we must beware of assuming it. The fact is that unless the control population is very carefully selected, there could easily be such a large variation in mortality in early life and life span determined environmentally, that its magnitude would totally mask anything that we could expect to find as being due to inborn differences.

Dr. Ademar Freire-Maia said that they were not considering any individual trait. Presumably, they were thinking in terms of trying to measure the effects of mutation in terms of viability, or more accurately relative mortality. The number of ways in which such variations can be measured in man seems to me rather limited, consisting essentially of life span, with particular reference to an earlier life period or age specific mortality.

There are a lot of reproductive losses which are presumably due to the mutation effects. Perhaps this can be measured in some way, but the opportunity of doing this with such small numbers does not seem particularly good.

Fertility may be a segregating " trait " or it may be multifactorial, but in either event, fertility differences in man, while possibly being of great biological importance can still be far too small to be detectable in any conditions, far less in these studies.

At one stage, I had a little difficulty in understanding Dr. Ademar Freire-Maia. I think he was emphasizing the difficulties in dealing with populations where the data were collected by family approach and that he was apprehensive about the overlapping between families and the difficulties of separating out relationships when they became complex, so that the effects might be counted twice. This is always a problem in investigations of this kind but it is readily overcome by patience and good methodology.

I think, perhaps, most human geneticists, when they are thinking in terms of reproductive losses, stillbirths, abortions, and so on, are, to an increasing extent, thinking as much in terms of the maternal genotype as the foetal genotype. One thing that could come from a carefully continued study, would be information, in a big community, of reproductive performances of sisters as compared with that of brothers. In regard to the chromosome studies, the time and effort required to do studies on chromosomes on such a scale is frightening. Dr. Doll and Dr. C. Brown have probably the biggest resources in the world for doing work of this kind, almost on a mass production basis, but they have not studied such large numbers as would be requested in this investigation.

Furthermore, if blood is going to be used, as in the present situation (bearing in mind the limitations of the present blood culture techniques), it would have to be carried out in the areas concerned, not by having the specimen brought back to an existing laboratory.

On the other hand, nuclear sex study is certainly simple to do, it would be of great interest, and could be done by the nurses. However we have no information as to the proportions of confinements in hospital, by doctors in homes and by midwives.

If Dr. Ademar Freire-Maia would like to answer these questions, if he thinks they are of interest, we can then open the meeting for general discussion.

Ademar Freire-Maia. I would like to answer succintly Dr. Stevenson's questions.

1) Children are generally born at home, with the assistance of a practical midwife. More wealthy families generally have a doctor to assist the delivery, at home or in the hospital.

2) The full data regarding the most recent Brazilian general census (1960) have not been published yet (obs. – at the time of the meeting).

3) Some gross medical data have been published, but it is my impression that different factors can account for eventual differences between populations. The discovery and interpretation of these factors probably can be attained only through a field work similar to ours.

4) I do not know at present the number of doctors and hospitals in each locality studied (*These figures have been obtained later*: cf 3.3. 9). Information regarding vital statistics, medical assistance, sex distribution, gross medical data, etc was not obtained previous to the field work, and even now some of the published data regarding such variables have not been consulted. Of course I fully agree with Dr. Stevenson in that such information could be of value as a first approach, both to selection of control populations and first crude comparisons. However, I did not want to have a preconceived idea when choosing the control populations and analyzing the data. This attitude is consistent with the general spirit which guided the project, namely, to minimize, as much as possible, the influence of subjective factors, both from people in general, the interviewers, and myself. (*Posteriorly on the basis of data published by the Instituto Brasileiro de Geografia e Estatística*, 1951, *the following values were estimated regarding sex ratio in the control, intermediate, and radioactive areas, respectively* 0.5027, 0.5040, *and* 0.5092. *The incidences of congenital blindness plus deaf-mutism in the same areas were found to be* 0.074%, 0.064%, *and* 0.064% *respectively*).

C. Pavan. It seems to me quite clear that the populations living in the region of Espírito Santo are not good enough for a project designed *only* to study the genetic effect of radiation. I think there is a general agreement about that. Now about the data obtained by Dr. Newton Freire-Maia in Minas Gerais, I would like to ask two questions:

1) Are the populations of Espírito Santo the most favorable for studies similar to those of Minas Gerais?

2) Can the results from Minas Gerais be tested in Espírito Santo?

Ademar Freire-Maia. I think there is a general interest in knowing the loads prevalent in different populations; in this respect, the genetic study of the populations from Espírito Santo is quite interesting. As the results from Minas Gerais can not be extrapolated to other regions, they can not be tested in Espírito Santo or in any other region, except, *perhaps*, in the areas neighboring the region studied in Minas Gerais (*This was done posteriorly by Dr. Newton Freire-Maia*). There is, however, a great interest in knowing whether the situation found in Minas Gerais is a local situation or is a phenomenon which can be found in other areas.

J. V. Neel. In a study of this nature, there are two alternative approaches, usually termed the prospective and the retrospective. The retrospective, which is the approach thus far applied to your situation in Brazil, is generally the more practical, although is in some respects theoretically inferior to the prospective. On the other hand, it is important to recognize how much greater an investment of time and effort is required to realize the theoretical superiority of the prospective study. In this particular instance, we must be prepared to think in terms of 10 to 15 years, plus a rather fair-sized organization.

In thinking about the desirability of large scale survey studies of this type, I find that I get a more honest answer from myself if I ask myself: is this a study to which I would devote 10 to 15 years of my life? In this particular instance, I would, of course, turn to the

experience of Dr. Schull and myself in Japan for guidance. In the follow-up study of the potential genetic effects of the atomic bombs, we examined altogether some 76000 children; about 6000 of these children were born to parents, one or both of whom were heavily irradiated. As a very rough figure, we can say the average combined dose to both parents would be in the neighborhood of 200 r. As you are aware, the only possible difference detected between these children and the control children, born to non-irradiated parents, involves a change in the sex ratio, and this is a small and still somewhat debatable finding.

Let us, in the light of this experience, look at your problem: the number of Brazilians living in this area of high background radiation is about 18000. Let us estimate that in the course of a 10-year prospective study, some 6000 children would be born to this population. You have estimated a cumulative dose to the gonad up to the mid-reproductive life of about 7 r. Assuming this population has been in this region for three generations, the cumulative gonad dose would amount to about 20 r. In round terms, the proposed study would seem to have about one-tenth of the resolving power of the study in Japan, and yet, as I have brought out, aside from the sex-ratio change, we have failed to detect any effect in our study there.

An additional factor to be kept in mind is that in Brazil we are concerned with low-level, chronic radiation which, from the standpoint of mutagenesis, appears to be less effective than the acute radiation experienced in Hiroshima and Nagasaki.

The only other place in the world where there is a similar situation to what we have been discussing here is, of course, Kerala State, India. In the interest of perspective, it is worth pointing out that the dose in Kerala State is perhaps four times as great as the average dose in Brazil, and the numbers of people involved several times greater.

It is true that from a properly organized study you will learn much of genetic interest in addition to data on possible radiation effects. However, I do not see that considerations of this type should be allowed to enter into the decision on the feasibility of studies in Espírito Santo.

O. Frota-Pessoa. We are very happy to see that Dr. Neel's opinion is the same as the opinion which Dr. Ademar Freire-Maia and the Brazilian Committee of Human Genetics expressed. Dr. Ademar Freire-Maia made clear in his report that the probability to get significant results regarding radiation effects is practically nihil. However, the project is an important one regarding other aspects of population genetics.

Ademar Freire-Maia. Unfortunately, Dr. Neel could not be present when I gave my report yesterday, but even in the working paper of the reports, which has been previously distributed to the participants of this Seminar, I clearly expressed the opinion that we did not expect to detect genetic effect of natural radiation in the areas studied.

In my report, I gave emphasis on the three main points of our project: (1) to get data of interest for genetic studies of human populations (this is assumed to be the most important aspect of the project); (2) to get data which could be of some value for an eventual prospective research; I would like to put special emphasis on the word *eventual* to show that, at least presently, we do not have any intention of performing such a long-ranged project; and (3) to get some data, imperfect though it may be, regarding the sensitivity of human genes to radiation. I think there was some misunderstanding, and I would like to recall once more that Brazilian geneticists do not intend to perform a long-ranged project.

C. Pavan. I would like to bring to mind that the project of the Human Genetics Committee, worked by Dr. Ademar Freire-Maia with the sponsorship of the Comissão Nacional de Energia Nuclear, was planned to get genetic information of these populations without much hope to detect genetic effect of radiations, on account of the size of these populations. We want only to establish some perspectives, not expecting to get positive results. Although all the arguments for not waiting positive results, it is important that the research be performed and that no effect be detected with such size of the sample and under such conditions. Besides other reasons there is the practical advantage of the populations living in those regions being less worried, since they are living under a climate of discussions and propaganda on the effect of radiation. In summary, I think that the research is justified, even though we know a priori that the possibility of getting positive results is small.

O. Frota-Pessoa. I would like to ask Dr. Neel what he thinks of the two papers published in the US, where levels of natural radiation are correlated to incidences of abnormalities.

J. V. Neel. The two questions at issue are: firstly, the accuracy of the data, and secondly, the interpretation of the data. As regards accuracy, yes, there do seem to be regional variations in malformation frequencies in the US. However, regional variations are known in other parts of the world, such as the differences in the frequency of anencephaly in Ireland and France, and here radiation is not at issue. With regard to interpretation, I can only point out that the slight differences in radiation level involved are only one of many possible factors which must be investigated. Furthermore, the actual radiation levels are so low that it seems highly unlikely, in view of everything we know about experimental radiobiology, that they could be causative in the differences observed.

C. Pavan. I would like once more to accentuate the aspect of the problem, which has been accentuated several times by Dr. Neel. In many instances, in human radiogenetics, the analysis of non-radiogenetics data is much more important for the comprehension of the problem than a research planned to specifically study the genetic effect of radiation in man. Therefore, the project Espírito Santo, performed in that state on account of the presence of monazitic sands there, can bring much more contribution to human radiogenetics through its indirect aspects regarding population structure, inbreeding effects etc, than from the possible direct data on the genetic effect of radiation.

J. V. Neel. I would like to ask what is the ethnic composition of the population under study, what are the numbers of people in each ethnic group, and to what extent is it a hybrid population?

Ademar Freire-Maia. As has been pointed out, the data are not yet analysed. Preliminary results showed that the frequency of foreign-born grand-parents of the couples interwiewed is equal to 14%. [*Posteriorly, on the basis of data published by the Instituto Brasileiro de Geografia e Estatística, 1951, the following values were estimated regarding the incidences of whites, mulattoes and negroes in the control (c), intermediate (i), and radioactive (r) areas, respectively*: (*c*), 55%, 24%, *and* 21%; (*i*), 64%, 19%, *and* 17%; (*r*), 58%, 23%, *and* 19%].

C. Pavan. What is the frequency of African grand-parents?

Ademar Freire-Maia. It is less than 0.6%, which is the frequency of " others ".

J. V. Neel. By the 86% who are not of foreign extraction, do you mean Indians? The point of my question was to bring out that this is a somewhat hybridized population.

Probably no human population is ever in genetic equilibrium, but hybridized populations are particularly far from it. The manner in which such populations might be expected to reflect increases in the mutation rates is very involved, as is the matter of suitable controls.

Newton Freire-Maia. The frequency of Indian descendants is extremely low in the samples obtained in this area.

Ademar Freire-Maia. By the 86% who are not of a foreign extraction I mean those who were born in Brazil. It is believed that the majority of them (especially the white fraction of the sample) are from Portuguese ancestry.

A. C. Stevenson (Presiding over the biogenetic working group).

I want to summarize the opportunities offered to our Brazilian colleagues.

Dr. Ademar Freire-Maia has organized a wonderful piece of work and achieved as a primary purpose, an exercise in methodology, namely how to classify retrospective data collected in specific areas. The second aspect of Dr. Ademar Freire-Maia's study is that, in the light of experience, he will now wish to extend his investigations. Following this, the data will be analysed in association with dosimetry and physical data. This is a big task, requiring hard work and intelligence.

After the present stage of Dr. Ademar Freire-Maia's inquiry, or after completion of an extension of it, it is, I understand, proposed to proceed to some kind of a prospective investigation, under no illusions of getting information which will give qualitative or quantitative data, or estimates associated with detriment to population living in these areas of HBR. It is this stage, which has not been exactly formulated. This is a difficult decision that Dr. Freire-Maia must make, but, clearly, a decision that must be made soon. A lot of preliminary work has been done on the physical side. Dr. Freire-Maia has worked hard, spent time, money, and effort, therefore the decision must be made soon in order not to waste the preliminary work.

It was quite clear from the remarks of our Brazilian colleagues that they have no illusions what-so-ever, as to the difficulties ahead, and none of us have any substantial difference of opinions as to the concrete results likely to be obtained.

The conclusion is that, with the number of people involved and the level of additional radiation received by these people, even if a perfect prospective study could be undertaken, it is unlikely that a positive result could be obtained, unless, as Dr. Neel pointed out, our whole conception of the relationship between detriment to population and radiation is quite wrong. As the Americans say, the areas of ignorance are enormous. It is not an encouraging project from which a young man could make his reputation.

When, in addition to those difficulties which Dr. Neel outlined so succintly yesterday, you dismiss the extremely difficult epidemiological situation in the area which must be investigated, then I think the gap between hope and the prospect of getting concrete results is even greater. I can barely conceive of a more difficult epidemiological investigation than one which makes it necessary to go to areas where vital statistics are rudimentary, where the standards of education are low, where there is not one area but a whole series of areas, where the control populations would have to be also in a series of separate areas, where there

are differences which have not been made quite clear to us, and where you have all of the socio-economic variables, industrial variables, racial variables, duration of stay in the areas, status variables, etc. It is the most difficult type of investigation for any one to conceive of undertaking.

The last possibility seems to me to be without prejudice to any of the preceding choices, or perhaps without starting a prospective investigation on a purely epidemiological basis, that you think in terms of evolving methods in the laboratory which will remove many of the difficulties in the observational questionary type of inquiry. Many laboratory techniques which seem very attractive, are quite unsuitable for adaptation to the epidemiological method in which they have to be employed. Techniques in the laboratory may be beautiful, efficient, repeatable, but unless they can be done very quickly on a scale large enough to allow specimens to be brought from long distances to a big laboratory, then the hundreds or thousands of such examinations which need be carried out, may make the method quite unsuitable for epidemiological investigations.

Let us take for example a technique Dr. Cordeiro mentioned yesterday, namely that of the rate of growth of tissue cells in culture according to the age of the person. What kind of discrimination do we want from this technique and in this particular situation in Brazil? On the analogy of mouse work we might expect one rad of whole body exposure to lower the life span of man by about one week. That would be only somatic effect; the genetic effect might double this. You are going to have an increment from background, 2-3 r, at most, in a large number of populations, but is it possible to have even an equivalent degree of discrimination using in vitro techniques in the laboratory? I doubt it. But there may be other methods.

You may not agree with my remarks, or there may be alternatives, but perhaps they will be helpful in clearing the air. I was asked this morning to comment on the specific proposals and told the visiting participants should make some recommendations. I am sure that the Brazilian members of this meeting may well wish us to do this, but I think it is asking too much of us, as we have little insight into the complexities of personal and political situations to make specific recommendations. The problems are rather, as we say in England, domestic questions. Thank you.

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