left. On June 23 bronchoscopy by Mr. A. H. M. Siddons showed the main carina to be distorted and widened; a swelling extended along the medial wall of the left main bronchus, which was narrowed slightly, and an irregular mass was seen protruding from the lower lip of the left

FIG. 2.—Section of bronchial biopsy specimen showing surface epithelium (on the left) and several dilated gland ducts surrounded by amyloid deposits (arrows). (Haematoxylin and eosin. $\times 75$.)

upper lobe bronchus. Biopsy specimens were taken from the swellings of the left main and left upper lobe bronchi. They showed bronchial epithelium and glands in connective tissue rich in amyloid deposits (Fig. 2). Characteristic reactions were obtained when the deposits were stained with both methyl violet and Congo red. There was no evidence of neoplasm. Bronchography on July 8 showed that the left upper lobe bronchus was occluded (Fig. 3). At

FIG. 3.—Bronchogram showing occlusion of the left upper lobe bronchus.

On July 14 the appearances were the same as those three weeks earlier, but several protuberances were removed with the biopsy forceps to widen the airway. On July 17 the patient was discharged from St. George's Hospital, but during November he again became ill with increased cough and dyspnoea, and his condition steadily deteriorated. On December 21 he was readmitted to St. George's Hospital. He was then gravely ill, mentally confused, cyanosed, and dyspnoeic. There was no response to antibiotic treatment, and he died on December 24. Necropsy showed that death was due to acute purulent bronchitis superimposed on chronic bronchitis and emphysema. Massive amyloid infiltration was present in the submucosa, muscularis, and adventitia of the left and right main bronchi. The deposit in the left main bronchus had made its wall up to 0.4 cm. thick. Histological examination of the liver, kidneys, and spleen did not show evidence of amyloid. The other organs did not show any significant disease.

Summary

The literature of amyloid tumours of the trachea and lung is reviewed briefly.

A case of amyloid deposits in the bronchi is described. The condition should be borne in mind when considering the nature of a bronchial tumour.

This case is published with the kind permission of Dr. A. L. Jacobs, physician, and Dr. A. B. Bratton, pathologist, both of Whittington Hospital, and Mr. A. H. M. Siddons, thoracic surgeon, St. George's Hospital.

REFERENCES

- Haynes, A. L., Clagett, O. T., and McDonald, J. R. (1948). *Surgery*, 24, 120.
Lunzenauer, K. (1952). *Frankfurt. Z. Path.*, 63, 519.
Noring, O., and Paaby, H. (1952). *Acta path. microbiol. scand.*, 31, 470.
Schottenfeld, A., Arnold, L. M., Gruhn, J. G., and Etes, A. D. (1951). *Amer. J. Med.*, 11, 770.
Whitwell, F. (1953). *Thorax*, 8, 309.

SIGNIFICANCE OF PHYSIOLOGICAL PATTERN OF ANIMAL STRAINS IN BIOLOGICAL RESEARCH*

BY

ELIZABETH S. RUSSELL, M.A., Ph.D.

Jackson Memorial Laboratory, Bar Harbor, Maine

Serious problems are presented in all biological research by differences in the characteristics and reactions of experimental and control animals caused by genetic and/or environmental variables. One large source of variation, genetic difference, can be practically eliminated by the use of inbred animals. Forty-two different inbred strains of mice are maintained and studied in the Inbred Nucleus of the Jackson Laboratory, Bar Harbor, Maine, U.S.A. The majority of mouse inbred strains were originally developed for cancer research, in which their first function was to prove the existence of genetic factors influencing the incidence of cancer and the independence of inheritance of different types of tumours. The same process of inbreeding which fixed the cancer-influencing genes in each of these strains fixed practically all of the 10^4 to 10^6 genes carried by any one strain. The individuals within each of the more than 100 inbred mouse strains which now exist are as alike genetically as it is possible to make and keep living material.

Classification

(on guinea pig.)
What types of characters are fixed in these strains? How do they differ from each other? The classification of strain differences given in Wright and Eaton's (1929) classic paper still does very well as a description of the type of differences between inbred groups. It is particularly valuable for its demonstration that genetic differences are very important even in cases in which they do not produce obvious effects under all conditions. Their first category, *complete fixation of a phenotype*, in cases in which the genes concerned have an all-or-none effect, might be exemplified for the mouse by the difference between white BALB/c mice, carrying the genes *cc* for albinism, and black C57BL mice, carrying the genes *CC* for intense colour. The second category, *fixation of average grade*, may be illustrated by comparing the incidence of mammary tumours in C3H females with 96%, DBA/1 females with 66%, and

*This investigation was supported by a research grant C-1074(C3) the National Cancer Institute, of the National Institutes of Health, U.S. Public Health Service. The paper is slightly altered from an address in the Proceedings of the Fourth Annual Meeting, Animal Care Panel, December 2 and 3, 1953, Chicago, Illinois, U.S.A.

C57BL/6 females with less than 1%.* These averages have remained fairly constant through the past 15-20 years.

Dr. Gowen, of Iowa State College, is responsible for the development and study of an excellent example of the third category, *fixation of relative rank*. He produced a series of mouse inbred lines differing in relative susceptibility to mouse typhoid (Gowen, 1950), the actual proportions succumbing depending, of course, upon the existence and extent of their exposure to *Salmonella* organisms. This category overlaps in some cases the fourth, *fixation of reaction to conditions*. The high susceptibility to sound-induced seizures characterizing the DBA strain and completely lacking in the C57BL strain (Fuller and Smith, 1953) is, however, an unequivocal example of this fourth category. A qualification which should be added to this classification has to do with a similarity rather than a difference. A considerable proportion of the animals in any generation of an inbred strain must grow to fertile healthy adulthood if brother-sister matings are to continue indefinitely. During inbreeding there will tend to be a reduction in fertility, since chance prevents accumulation of all favourable genes in one package. However, genes causing all or a high proportion of individuals to have severe *early* abnormalities could not be fixed in a strain. Genes leading to a high proportion of serious deleterious effects appearing *late*, after the production of a number of litters, have, however, been fixed in many strains. These effects include various cancers as well as other types of disease. Thus in all inbred strains extremely severe genetic effects cannot appear very early in the life span.

Pulling from a hat four type-examples of characters differing among inbred strains of mice would do little to convince a jury that the use of inbred animals is essential to most types of animal research. The judges may feel that coat-colour, tumour susceptibility, typhoid resistance, and reaction to loud noises have nothing to do with the problems faced by most investigators. It is hoped that presentation of a few further examples garnered from recent literature may give some indication of the probable extent of genetic influence upon physiological characteristics. In the library of the Jackson Laboratory there are more than 1,200 references, from 1951 to 1953 journals, to studies based on inbred mice. These have been catalogued and classified in a subject-strain bibliography (Staats, 1954) which provides rapid access to literature pertinent to a particular strain or condition. For the purposes of this presentation I have listed some of the newer physiological differences among strains gleaned from this bibliography, but I have omitted from consideration a much larger number where a single strain was used simply to provide genetic uniformity or because previous work had shown it to be the best for a particular purpose.

Strain Differences in Reactions

Different strains have been shown to react very differently to several external environmental factors. The *nature of disease* produced by a certain pathogen may depend upon the genotype of the host. For example, the Newcastle virus usually causes pneumonia when administered intranasally to DBA/2 and C3H mice, but produces encephalitis in DBA/1 mice (Liu and Bang, 1952). Genetic difference may alter the *survival time* of individuals infected with a certain

pathogen. The average survival of DBA/2 mice after standard inoculation with the malaria-producing *Plasmodium berghei* is 9.9 days, of C57BL mice 13.2 days, and of their hybrid DBA×C3H F₁, 19.7 days (Greenberg *et al.*, 1953). The longer survival of the hybrid is due to its greater ability to tolerate parasitaemia rather than to delay in infection. Certain strains of mice are frequently plagued with diseases which never hit mice in adjacent pens. The infantile diarrhoea of C3H mice (Runner and Palm, 1953; Russell, 1954) is an example of this type of *strain-limited susceptibility*. It may hit as many as 50% of litters born in winter months, and is especially frequent in first litters.

Strains differ in their capacity for *antibody production* (Fink and Quinn, 1953). Antibodies against protein were produced in five strains by intramuscular injection of egg albumen. Animals of the highest strain, DBA, were more than four times as reactive as those of the lowest strain, BALB/c. Injection of pneumococcus polysaccharide also showed significant differences among the same five strains, but the order of reactivity is very different. A/Lilly, the most sensitive, was four times as reactive as C57BL/6.

Experiments which involve taking research animals to outlying districts have created a necessity for finding animals which can tolerate very low temperature and sudden change. Weanling BALB/c mice have such *cold tolerance*, and when put outdoors in winter can easily survive for 30 days, including periods of 0° F. temperature, while C57BL mice under the same conditions die in less than a single day (Curran, 1955). On at least three occasions serious loss to animal colonies has resulted from fumes of chloroform or allied products (Deringer *et al.*, 1953; Shubik and Ritchie, 1953). Males only of particular strains are affected. In a Jackson Laboratory catastrophe more than 74% of DBA/2, 51% of DBA/1, 32% of C3H, and 10% of BALB/c breeding males died within a week with a characteristic *toxic destruction* of the kidney. This event had no effect on females of these strains or on large colonies of six other strains in the same room.

It is clear that variations in reactions to external agents such as have been described above could wreck an experiment using non-homogeneous animals. It is also clear that there are "strains of choice" for particular experiments. There is also considerable evidence that the endocrine picture varies with genotype in a way which may interfere with interpretation of experimental results. It has been shown that strains differ in their *content of particular hormones*. Evidence has been presented indicating the gonadotropin content of DBAs is 1.5 times that of mice of the A or 020 strains (Mühlbock *et al.*, 1952). The thyroxine level of C3H appears to be approximately 1.5 times that of C57BL (Gross and Schwartz, 1951). Strains also vary in their *sensitivity to particular hormones*. It has been shown that the C57 group of strains is approximately 12 times as sensitive as the DBA, BALB/c, or 129 strains in its eosinophil response to adrenal cortical hormones (Wragg and Speirs, 1952). The type of reaction of different tissues to a particular hormone varies with strain. The susceptibility to oestrogen of breast, vaginal epithelium, and endometrium of three strains has been compared (Silberberg and Silberberg, 1951). The Silberbergs reported the mammary tissue of DBA and A more susceptible to oestrogen than that of C57BL, and the vaginal epithelium and endometrium of C57BL very sensitive, of DBA intermediate, and of A strain relatively resistant. Recent work by Drasher (1955) indicates that the strain difference in uterine reaction may be one of duration of reaction rather than of initial susceptibility.

Strains of mice differ qualitatively in their *reaction to extirpation* of endocrine organs. Removal of ovaries from day-old C57BL mice does not alter the fate of their adrenals. The same operation on DBA females eventually leads to nodular hyperplasia of the adrenal, with secondary effects on other endocrines. In CE females the same operation results in adrenal cortical carcinomas. Recent work

*Inbred Nucleus means animals born 1948-50.

on the inheritance of these differences (Woolley *et al.*, 1953) has shown 100% adrenal cortical carcinomas in the F₁ hybrids of both C57BL and DBA with CE.

At present a rapidly expanding field of study is strain difference in enzyme activity. In this field I am not qualified as an expert, but can at least list enzymes which have been studied. For example, within the past three years differences among strains have been described in liver β -glucuronidase (Law *et al.*, 1952; Fishman and Farmelant, 1953; Sie and Fishman, 1953), mammary glucuronidase, and esterase (Cohn and Bittner, 1951), serum tributyrinase (Tuba, 1952), and serum alkaline phosphatase (Tuba, 1953). The work on probable mode of inheritance of difference in liver glucuronidase activity, which is less than one-tenth as high in C3H as in A strain mice (Law *et al.*, 1952), is particularly impressive.

Physiological Strain Differences

In addition to the Jackson Laboratory's attempt to keep up with the growing knowledge of the characteristics of inbred strains by following the literature, certain observations have been carried out on the animals maintained in the Inbred Nucleus of the Jackson Laboratory which indicate physiological strain differences. A study of the formed elements of the blood of young adults from 18 different inbred strains, for example, showed that differences among strains contributed very significantly (0.1% level) to variations in *erythrocyte number*, *haematocrit*, and *haemoglobin*, even though there were great overlaps between strains (Russell *et al.*, 1951). The mean haemoglobin content varied from 12.2 g.% for C3H/Jax to 15.0 g.% for BALB/cJax. Strain differences in leucocyte characteristics were also very conspicuous in spite of the wide variation within strains to be expected in a character so easily influenced by environmental factors. The mean total leucocyte counts for the various strains ranged from 5.87×10^3 for RIII/Jax to 11.43×10^3 for C57BL/6Jax, and strain differences contributed very significantly (0.2% level) to the total variance. The differential leucocyte count also varies significantly with strain (0.1% level), with the entire C57 group of strains very low in granulocyte percentage. Subsequent work has shown that different genes affect granulocytes and agranulocytes (Budds *et al.*, 1953), since the rank order for granulocyte number (C3H>DBA/1>BALB/c>DBA/2>A>AKR>C57BL/6) is very different from that for agranulocyte number (C57BL/6>DBA/1>BALB/c>DBA/2>A>C3H>AKR).

In the Inbred Nucleus breeding mice are retired at 1 year of age, but they are allowed to live out their life-span for

in survival in mammary tumour strains also varies with the strain. The differences among means of males of all strains and females of the low mammary strains show that factors other than mammary tumour susceptibility strongly affect survival, so that strain means can run from approximately 280 days for the AKRs, which usually die with lymphatic leukaemia, to approximately 550 days for C57BL/6. In low mammary strains, under the conditions of our colony, females tend to outlive their mates slightly, although there are exceptions. It is clear that ageing processes must vary greatly with strain. It is hoped that analysis, now under way, of necropsy data collected on these strains will increase our knowledge of patterns in mouse gerontology. Whatever the causes behind these genetic ageing differences, their existence could lead to erroneous results from long-term experiments involving heterogeneous animals, and could dictate the choice of inbred animals to fit the time requirements of particular experiments.

Importance of Genetic Differences

The literature examples on endocrine balance and response and on enzyme activity, plus the Inbred Nucleus examples on blood picture and longevity pattern, show that there must be great differences in internal constitution and relationships influenced by genes. As with the reactions to external agents, such differences *within* an experiment could lead to fallacious conclusions. If we could only come to know each strain well enough, such differences between groups could be invaluable tools. There is so very much to know. The million or so genes carried by a single mouse may have direct or indirect effects on many characters. The nature of effect of any particular gene may depend a great deal on its interaction with other genes. We have seen that genetic differences do not always have an all-or-none effect, but may alter average grade, relative rank, or reaction to conditions.

This means that two genetically different individuals may under some conditions be identical for a given character, but under other circumstances will differ. It also means that there are variations within as well as between inbred strains. The recent rapid increase in examples of strain differences means that more and more investigators are coming to realize the importance of genetic difference and are beginning to tap the mine of information that is waiting for them in the already established inbred types. Many of them have started their experiments to test a hypothesis on the basis for a known strain difference, as the incidence of a certain type of tumour (Gross and Schwartz, 1951; Silberberg and Silberberg, 1951; Mühlbock *et al.*, 1952; Greenberg *et al.*, 1953), but more often than not they have turned up what appears at present to be a completely independent attribute of the strain difference. Other investigators have accepted the extremely valuable concept of "strains of choice" for a given experiment, and start their investigations with a cross-strain test of their particular procedure or substance. This is an extremely wise course of action, since they eventually obtain both suitable and genetically homogeneous material. Unless they are theoretical geneticists, however, their interest ends with establishing their base-line.

Since this is a paper on the significance of physiological pattern of animal strains, it seems suitable to mention the great importance of variations within strains to students of the relation between heredity and environment. Analysis of association of intra-strain differences with life-history differences, including parental effects, is the most valuable tool in this field (Wright and Eaton, 1929; Runner and Palm, 1953; Russell, 1954). For characters with great intra-strain variation, greater variation between strains often provides geneticists with the only evidence that genes have anything to do with the character (Russell *et al.*, 1951; Budds *et al.*, 1953).

Thus the rapidly increasing knowledge of inherited differences between strains in their reaction to conditions, their internal balance, and their normal levels of certain cells or

Mean Life-span of Male and Female Mice from the 10 Largest Inbred Colonies

Strain	Mean Life-span (Days)	
	Females	Males
<i>High-moderate Mammary Tumour Strains</i> (in order of decreasing incidence)		
C3H	314.4	424.3
DBA/1	399.4	411.5
DBA/2	431.0	401.0
A/He	389.2	453.8
<i>Low Mammary Tumour Strains</i>		
A/Jax	447.8	467.4
AKR	284.2	272.2
BALB/c	485.0	475.4
C57BL/6	562.7	529.4
C57BR/cd	482.9	457.2
C57L/He	513.5	490.6

the study of their potentialities for specific types of disease. The Table compares the mean life-span of males and females from the ten largest inbred colonies, using animals born during a three-year period, 1948-1950 (Russell *et al.*, 1955). Several conclusions can be drawn from these data. They confirm the previous observation that mammary tumours strike at different ages in different strains. As a general rule, the longevity of females in mammary tumour strains is less than that of males, but there is one exception in our data, in DBA/2. The extent of the sex-differential

substances makes it more and more apparent that genetic homogeneity is essential to most physiological work, and that genetic variety between strains provides an array of valuable tools for particular types of physiological experiments.

Summary

The use of inbred strains in biological research is essential for fixation of either phenotype, average grade, relative rank, or reaction to conditions. Examples are presented of each type of strain differences in mice, gleaned from 1951-3 literature and from research in the Inbred Nucleus of the Jackson Laboratory. These include differences in disease susceptibility, nature of disease produced by a given pathogen, survival time of infected individuals; capacity for antibody production, cold tolerance, reaction to specific toxins; sensitivity to and content of various hormones and reaction to endocrine extirpation; and differences in normal blood-cell levels, life-expectancy, and pathology pattern.

REFERENCES

- Budds, Olga C., Russell, E. S., and Abrams, Gerald E. (1953). *Proc. Soc. exp. Biol. (N.Y.)*, **84**, 176.
Cohen, S. L., and Bittner, J. J. (1951). *Cancer Res.*, **11**, 723.
Curran, R. H. (1955). *Genetics*, **39**. In press.
Deringer, Margaret K., Dunn, Thelma B., and Heston, W. E. (1953). *Proc. Soc. exp. Biol. (N.Y.)*, **83**, 474.
Drashner, Marion L. (1955). *J. Hered.* In press.
Pink, Mary A., and Quinn, Virginia A. (1953). *J. Immunol.*, **70**, 61.
Fishman, W. H., and Farnelant, M. H. (1953). *Endocrinology*, **52**, 536.
Fuller, J. L., and Smith, Mary E. (1953). *Amer. J. Physiol.*, **172**, 661.
Gowen, J. W. (1950). *Genetics in the Twentieth Century*, edited by L. C. Dunn, p. 401. MacMillan, New York.
Greenberg, J., Nadel, E. M., and Coatney, G. R. (1953). *J. infect. Dis.*, **93**, 96.
Gross, J., and Schwartz, S. (1951). *Cancer Res.*, **11**, 614.
Law, L. W., Morrow, A. G., and Greenspan, E. M. (1952). *J. nat. Cancer Inst.*, **12**, 909.
Liu, Ch'ien, and Bang, F. B. (1952). *Amer. J. Hyg.*, **55**, 182.
Mühlbock, O., van Ebbenhorst, Tenzbergen, W., and van Maurik, G. (1952). *Acta endocr. (Kbt.)*, **9**, 48.
Runner, M. N., and Palm, Joy (1953). *Proc. Soc. exp. Biol. (N.Y.)*, **82**, 147.
Russell, E. S. (1954). *Ann. N.Y. Acad. Sci.*, **57**, 597.
Neufeld, E. F., and Higgins, C. T. (1951). *Proc. Soc. exp. Biol. (N.Y.)*, **78**, 761.
Fekere, Elizabeth A., Borges, Paulo R. F., MacFarland, Eleanor K., and Collins, Joan B. (1955). *J. exp. Zool.* In press.
Shubik, P., and Ritchie, A. C. (1953). *Science*, **117**, 285.
Sie, H'sien-gieh, and Fishman, W. H. (1953). *Cancer Res.*, **13**, 590.
Silberberg, M., and Silberberg, Ruth (1951). *Proc. Soc. exp. Biol. (N.Y.)*, **76**, 161.
Staats, Joan (1954). *Science*, **119**, 295.
Tuba, J. (1952). *Cancer Res.*, **12**, 113.
— (1953). *Canad. J. med. Sci.*, **31**, 41.
Woolley, G. W., Dickie, M. M., and Little, C. C. (1953). *Cancer Res.*, **13**, 231.
Wragg, L. E., and Speirs, R. S. (1952). *Proc. Soc. exp. Biol. (N.Y.)*, **80**, 680.
Wright, S., and Eaton, O. N. (1929). *Tech. Bull., U.S. Dep. Agric.*, **103**, 1.

The Singapore Anti-tuberculosis Association has recently published a well-illustrated account of the origin and activities of the Royal Singapore Tuberculosis Clinic, which has been in existence since 1948. In a foreword to the book the Right Hon. MALCOLM MACDONALD, Commissioner-General for the United Kingdom in South-east Asia, refers to the great increase in the work of the clinic, and continues: "Despite this attack, tuberculosis still remains a vast and dangerous problem in Singapore. Just how vast and how dangerous is still far from understood by the general population." The figures given in this book should dispel complacency. There may be as many as 50,000 patients needing treatment for tuberculosis in Singapore. Every day at the Royal Tuberculosis Clinic, which is a voluntary organization, some 20 new cases of tuberculosis are discovered—a steady 8 to 9% of those x-rayed each month. In all, nearly 60,000 radiographs are taken every year and 55,000 blood sedimentation tests performed. Between three and four thousand patients are under treatment at any one time, and in 1953 the total of patient attendances was over 150,000. The work of the clinic is carried on in two separate units, one for diagnosis and the other for treatment. There are no beds, but domiciliary treatment has proved remarkably successful, particularly among the Chinese. A children's clinic has now been started, where some 130 children a month are brought by their parents.

PELVIC TUBERCULOSIS

A REPORT ON 19 CASES

BY

ALEX KOUYOUMDJIAN, M.D.

American University Hospitals, Beirut

Morgagni (quoted by Norris, 1931), in the eighteenth century, was the first to recognize pelvic tuberculosis, but no further mention of the disease is found in the medical literature until 1831. Since that time numerous reports have appeared. The recent advent of streptomycin, P.A.S., and isoniazid has stimulated a new interest among gynaecologists.

The incidence of pelvic tuberculosis in the female is said to vary in different parts of the world. Sharman (1952), in Glasgow, and Rabau (1950), in Israel, have reported a high incidence of the disease in their respective clinics. I consider that pelvic tuberculosis in the Lebanon is more common than is generally believed, and this report is the first of its kind from that country.

Material

A review of the records at the American University Hospitals, Beirut, revealed 35 cases listed under the heading of pelvic tuberculosis for the seven-year period July 1, 1946, to June 30, 1953. However, a careful study of the case records shows that the diagnosis was established histologically in only 19 cases, and this study is confined to these 19 proved cases. The age incidence is shown in Table I.

TABLE I.—Age Incidence

Age	No. of Patients	Percentage
10-19	0	0
20-29	7	36.8
30-39	6	31.6
40-49	4	21.1
50-59	2	10.5
Total	19	100

Nulliparae, 8 (42%); average age, 28.6 years. Multiparae, 11 (58%); average age, 36.6 years.

During the same seven-year period there were 2,492 admissions to the gynaecological service of this institution. Thus the incidence of pelvic tuberculosis in these 2,492 admissions was approximately 0.8%. This is considerably lower than Leon's (1950) figure of 2% for all gynaecological conditions. Greenberg (1921) gives the incidence of tuberculous salpingitis among 24,155 gynaecological admissions to the Johns Hopkins Hospital as 0.83%.

Symptoms

Abdominal Pain.—The commonest symptom encountered in this series was low abdominal pain. This was present in 57.8% of the cases. Typically, the pain was of a dull nature, beginning in one of the lower quadrants and later being bilateral. Acute abdominal pain with fever simulating acute salpingitis or appendicitis has also been reported. Usually the attacks of pain became worse during the menstrual periods, but dysmenorrhoea *per se* was the admission complaint in only 15.8% of our cases. Backache was present in 27.4%.

Sterility.—The next commonest complaint was sterility (52.7%). It was primary in 42.2% and secondary in 10.5%. These figures are in sharp contrast to those given by Brown *et al.* (1953), who report it in only 6.9% of their cases. Greenhill (1951) states that tuberculosis is an infrequent cause of sterility, but in some parts of the world 5 to 6% of all endometrial biopsies performed on infertile women show tuberculosis. Sharman (1952) found histologically proved endometrial tuberculosis in 5.2% of 2,985 patients