The Influence of Progressive Growth on the Specific Catalase Activity of Human Diploid Cell Strains

II. EFFECT OF CELLULAR GENOTYPE: A HETEROZYGOUS STRAIN

YUE-LIANG PAN, ROBERT S. KROOTH AND SHIGEO TAKAHARA

Lawrence D. Buhl Center for Human Genetics, Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan and Department of Oto-Rhino-Laryngology, Okayama University Medical School, Okayama, Japan

ABSTRACT Human diploid cell strains develop progressively higher levels of specific catalase activity as they grow. Following subculture activity falls again. A diploid cell strain heterozygous for the gene for acatalasia I (acatalasemia) was found to develop specific catalase activity at *proportionately* the same rate as normal cell strains. Yet the mutant gene reduced the absolute level of specific catalase activity which the culture attained at any given point in time. In this respect the heterozygous acatalasia I strain resembles the homozygous acatalasia II strain previously reported.

In our previous paper, (Pan and Krooth, '68) two of us showed that the specific catalase activity of human diploid cell strains increases as the culture grows. Specific activity falls again immediately after subculture. Although the increase is not large, it is exponential with time and repeatable. A cell strain from a donor who was homozygous for the gene for the Swiss form of acatalasia (acatalasia II) had a specific catalase activity which was about 5% that of normal diploid strains. A similar result was obtained by Aebi, Baggiolini, Dewald, Lauber, Suter, Micheli and Frei, '64. The acatalasia II cells, like the normal cells, increased their specific activity as they grew. The *proportionate* increase in specific catalase activity with time was the same as that of normal cells. At every point in the growth cycle, the acatalasia II cells had about 5% the normal activity.

A cell strain from a patient who was homozygous for the Japanese variety of acatalasia (acatalasemia or acatalasia I) had no detectable activity, by the assay method employed, throughout its growth cycle. In this paper we wish to report the kinetics of development of specific catalase activity in a cell strain from a patient who was heterozygous (Cc_1*) for actalasia I.

MATERIALS AND METHODS

The heterozygous strain (HYC) was grown from a biopsy performed by one of us (Shigeo Takahara) on an adult Japanese male. Other materials, methods, and notation are described elsewhere (Pan and Krooth, '68).

RESULTS

Figure 1 describes an experiment measuring the kinetics of growth and of development of specific catalase activity in the heterozygous HYC strain. Note that in this diploid cell strain, as in the others previously reported, specific catalase activity increased, following an initial fall, as the culture grew until the plateau phase of growth was reached. Figure 2 gives the growth curves for two experiments (including the one in fig. 1) on the kinetics of development of specific catalase activity. The initial population densities in these two experiments were respectively 0.011 and 0.018 mg of cell protein per 18.7 cm^2 of surface area available for growth, which is about the same as that used with other cell strains, previously published, (Pan and Krooth, '68) with which we shall be comparing this strain. A doubling time of

Received Jan. 4, '68. Accepted Apr. 8, '68.

[1] These experiments are described in greater detail in our previous publication (Pan and Krooth, '67).

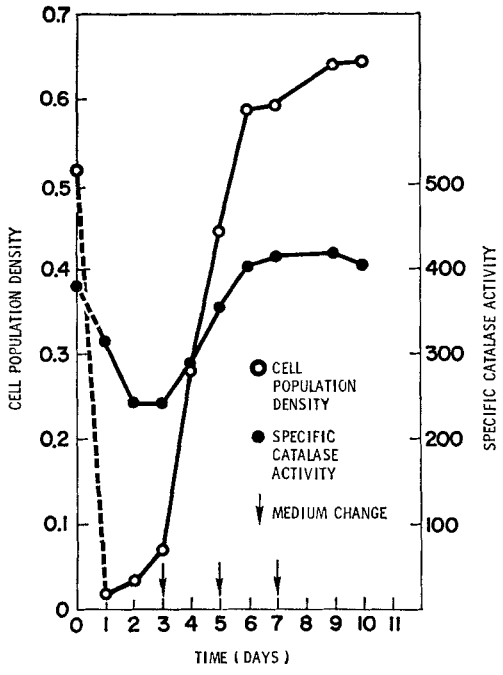

Fig. 1 Effect of progressive growth on the specific catalase activity of a diploid cell strain (HYC) heterozygous (Cc_I*) for acatalasia I.

about 24 hours was observed in both experiments with the HYC strain, while the other strains in which the kinetics of development of specific catalase activity was studied were growing with a doubling time of about 33 hours (Pan and Krooth, '68).

Figure 3 summarizes three experiments measuring the kinetics of development of specific catalase activity using two diploid cell strains with normal catalase levels,[1] two experiments on an acatalasia II strain,[1] and the two experiments on the heterozygous HYC strain. Specific catalase activity is plotted on a logarithmic scale and time on an arithmetic one. Note that on the semilogarithmic plot the kinetics of development of specific catalse activity by the HYC strain (and by the other strains) is linear. The *proportionate* increase in specific catalase activity with time is about the same in all the strains. The level of catalase activity at each point in time seemed to show more variation between experiments in the case of the heterozygous strain than in the case of the homozygous ones.

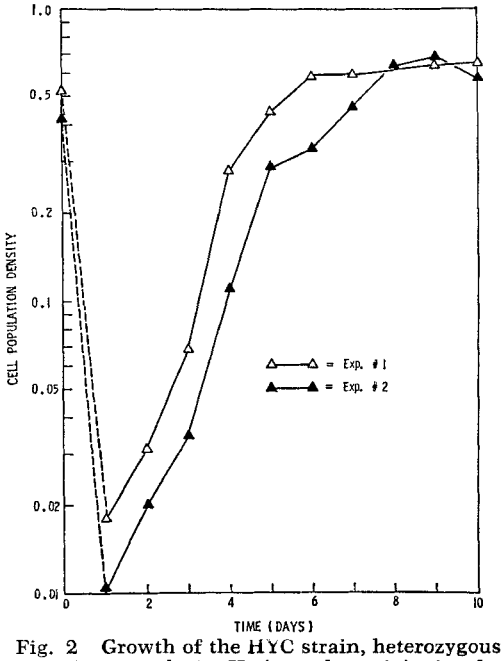

Fig. 2 Growth of the HYC strain, heterozygous (Cc_I*) for acatalasia II (acatalasemia), in the two experiments with this strain summarized in figure 3.

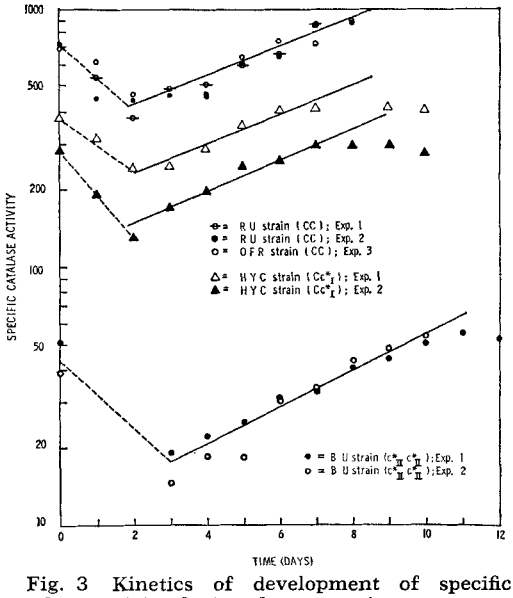

Fig. 3 Kinetics of development of specific catalase activity during three experiments on two cell strains homozygous (CC) normal for the catalase phenotype, two experiments on a cell strain (HYC) heterozygous for (Cc_I*) acatalasia I (acatalasemia) and two experiments on a cell strain (BU) homozygous (c_{II}* c_{II}*) for acatalasia II.

DISCUSSION

These data suggest that heterozygosity for the gene for acatalasia I, like homozygosity for acatalasia II, does not alter the kinetics of development of specific catalase activity in human diploid cell strains. Both abnormal genotypes, however, affect the absolute level of specific catalase activity which the culture attains at any given point in time.

ACKNOWLEDGMENT

The authors would like again gratefully to acknowledge the generous assistance of Professor Hugo Aebi, of the University of Zurich, who sent us the tissue from which the BU strain was developed.

These investigations were financed by area grant GM 09252 from the National Institutes of Health, United States Public Health Service.

LITERATURE CITED

Aebi, H., M. Baggiolini, B. Dewald, E. Lauber, H. Suter, A. Micheli and J. Frei. 1964 Enzymol. Biol. Clin., *4:* 121–151.

Pan, Y-L., and R. S. Krooth 1968 The influence of progressive growth on the specific catalase activity of human diploid cell strains. I. Effect of cellular genotype: Homozygous strains. J. Cell. Physiol., *71:* 151–160.