The Search for Anticancer Antibiotics

Some Theoretical Problems

G. F. Gause

Although the empirical approach is inevitable in the search for anticancer antibiotics, the efficiency of the screening increases when one combines empiricism with the theoretical analysis of specific metabolic differences between normal and malignant cells and utilizes these differences for the detection of substances that selectively inhibit those aspects of metabolism which are peculiar to the cancer cell.

As is well known, much work still remains to be done by the biochemists on the analysis of the specific metabolic differences between normal and malignant cells. It seems likely, however, that hereditary injury of the respiratory system of the cancer cell is one of the established characteristics of malignant growth. This impairment was first observed by Warburg about thirty years ago and, in spite of some criticism, has been entirely substantiated by more recent studies (*1, 2*). Measurements of the metabolism of malignant human cells show that hereditary deficiency of respiration is sometimes accompanied by a hereditary decrease, and sometimes by an increase, of aerobic glycolysis in the various strains of cancer cells (*3*).

It is to be recalled that alterations of metabolism peculiar to malignant growth can take place in cells of various living organisms. Julian Huxley was right in his recent generalizations (*4*) that "cancer is not merely a medical problem; it is a biological phenomenon, whose elucidation is bound up with advances in a number of key fields of present-day biology. . . . Neoplastic tumours are widespread in the organic realm."

The author is professor in the Institute for Antibiotic Research of the Academy of Medical Sciences of the U.S.S.R., Moscow. This article is based on a paper presented in New York on 21 Nov. 1957 at the Pasteur Fermentation Centennial sponsored by Chas. Pfizer & Co., Inc.

If Huxley is correct, could one not obtain some equivalents of cancer cells in microbiology? That is, could one not obtain biochemical mutants of microorganisms with hereditary impairment of their respiratory system and, most important of all, utilize these mutants as tests in screening for antibiotics that selectively inhibit malignant growth?

Microorganisms with Injured Oxidation

Some studies in this direction have been recently made in the Institute for Antibiotic Research of the Academy of Medical Sciences in Moscow. The first stage of investigations consisted in the production of biochemical mutants of microorganisms with injured oxidation. Gause, Kochetkova, and Vladimirova (*5*) used for this work yeast cells, *Saccharomyces cerevisiae* strain AN-2. Under the action of trypaflavine, which, according to Ephrussi and his collaborators (*6*), induces in yeast mutations with a deficient cytochrome system, we obtained six different mutant strains. The data on the respiration and aerobic glycolysis of these strains are given in Table 1. In all six mutant strains studied by us there was a strong hereditary injury of the respiration; the oxygen consumption in various mutant strains was decreased to 1/10 to 1/200 of that of the parent cells. It is interesting to record in this connection that, in some mutants, the injury to respiration was accompanied by an increase of aerobic glycolysis (strains 7, 22, and 25); in others, aerobic glycolysis was only slightly enhanced (strains 11 and 26); and finally, in one of the mutant cultures (No. 33), the aerobic glycolysis was decreased as compared with the value characteristic for the parent culture. In other words, in biochemical mutants of yeast with impaired oxidation, aerobic glycolysis can change in different directions, in the same way as it was observed in studies of aerobic glycolysis of malignant cells belonging to different strains. It is only now, one hundred years since Pasteur began his work in this important field, that we come to the understanding that, in biochemical mutants, aerobic glycolysis can change independently of alteration in the respiratory system.

Furthermore, we obtained mutant strains of yeast with injured respiration under the action of ultraviolet radiation and of camphor; the latter, according to Bauch (*7*), possesses strong mutagenic action upon yeasts. The data on the respiration and aerobic glycolysis of these strains are presented in Table 1.

For the rapid detection of the biochemical mutants of yeast cells with deficient oxidation, we worked out a specific method of staining their colonies, enabling us to distinguish by color the colonies of mutants from those of the normal cells. The leucobase of methylene blue was used for this purpose. Roskin (*8*) observed that normal human cells rapidly oxidize the colorless leucobase and stain themselves with deep blue color, whereas cancer cells under the same conditions of experiment remain colorless because of their deficiency in respiration. We observed that this principle of differential staining can be employed for diagnostic purposes to detect the biochemical mutants of yeast cells with impaired respiration, if the leucobase of methylene blue is added to the beer-wort agar media before the latter is poured into the petri dishes. The growing colonies of normal yeasts acquire deep blue color, whereas the colonies of mutants remain colorless, to the extent that they are unable to oxidize leucobase into methylene blue because of the deficiency of their respiratory system.

Besides yeast cells, Gause, Kochetkova, and Vladimirova (*9*) also used staphylococci for producing biochemical mutants with deficient respiration. The staphylococci turned out to be particularly useful as tests for the detection of substances possessing selective inhibitory action upon cells with an impaired respiratory system. Under the action of ultraviolet radiation of *Staphylococcus aureus* strain 209, we obtained three mutant cultures, Nos. 1, 2, and 3, which differed from the original parent strain by the small size of their colonies, by a de-

creased growth rate, and by a hereditary deficiency of respiration, as is shown by the data presented in Table 2. Table 2 shows that, in different strains of mutant staphylococci, the consumption of oxygen decreases to 40 to 65 percent of that of the parent culture. The decrease of oxygen consumption in the biochemical mutants of staphylococci is not as great as it is in the biochemical mutants of yeasts, in which oxidation, as is shown by the data of Table 1, may sometimes decrease to 1/200 of that of the parent culture—that is, it is practically excluded under aerobic conditions. As is well known, the consumption of oxygen in cancer cells may often decrease to half of the value characteristic for normal cells. In other words, the impairment of the respiratory system of cancer cells is quantitatively more like that of biochemical mutants of staphylococci than it is like the respiratory deficiency in the mutant strains of yeasts.

Some biochemical differences between mutant strains of staphylococci Nos. 1, 2, and 3 will not be considered at present. Let me only note that the respiration of all these mutant strains is much less sensitive to cyanide than is the respiration of the parent culture. This fact points to a deficiency of the cytochrome system as a cause of impaired respiration in the mutant strains.

Use of Mutants for Testing

Having outlined briefly some characteristics of the biochemical mutants of yeast and staphylococci with impaired respiration which have been obtained by us, we can now pass on to what seems to be the essential part of the investigation —namely, the use of these mutants as tests in screening for antibiotics that selectively inhibit cells with impaired respiratory systems. For this purpose, 2500 different cultures of actinomycetes freshly isolated from various soils were streaked upon nutritive agar in petri dishes, and after 48 hours of growth at 28°C they were cross-streaked by suspensions of different microorganisms, including parent cultures and mutant strains of staphylococci and yeast cells. According to the data obtained by T. P. Preobrazhenskaia and E. S. Kudrina (*10*), 53 cultures (that is, about 2 percent) possessed selective inhibitory action upon the biochemical mutants of staphylococci with impaired oxidation. These cultures did not inhibit the growth of

Table 1. Respiration and aerobic glycolysis in biochemical mutants of *Saccharomyces cerevisiae* with impaired oxidation (from Gause, Kochetkova and Vladimirova, *5*). Q_{O_2} shows the consumption of oxygen in cubic millimeters per hour, and Q_{CO_2} gives the production of carbon dioxide in cubic millimeters per hour, calculated for 1 milligram of the dry weight of yeast.

Culture	Respiration		Aerobic glycolysis	
	Number of experiments	Q_{O_2}	Number of experiments	Q_{CO_2}
Control (parent)	19	41.8	24	326.0
Trypaflavine 7	4	4.2	6	427.0
Trypaflavine 11	4	5.1	4	388.0
Trypaflavine 22	4	0.2	6	414.0
Trypaflavine 25	4	0.2	4	411.7
Trypaflavine 33	4	6.0	4	282.0
Trypaflavine 26	4	5.2	4	360.5
Camphor 27	4	4.2	2	339.3
Camphor 4	4	3.5	2	386.0
Camphor 23	4	10.5	4	353.0
Camphor 2	4	10.9	4	410.0
Ultraviolet 10	8	2.9	8	321.0

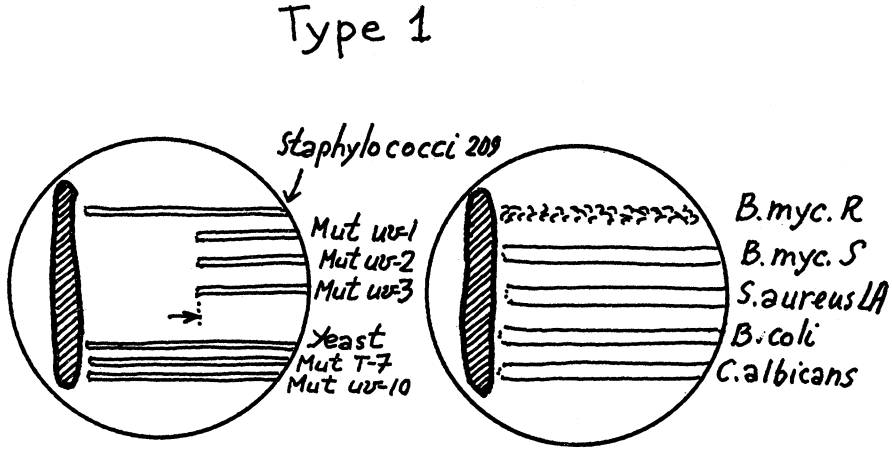

Fig. 1. Selective inhibitory action of a culture of actinomycete upon mutant staphylococci with impaired oxidation.

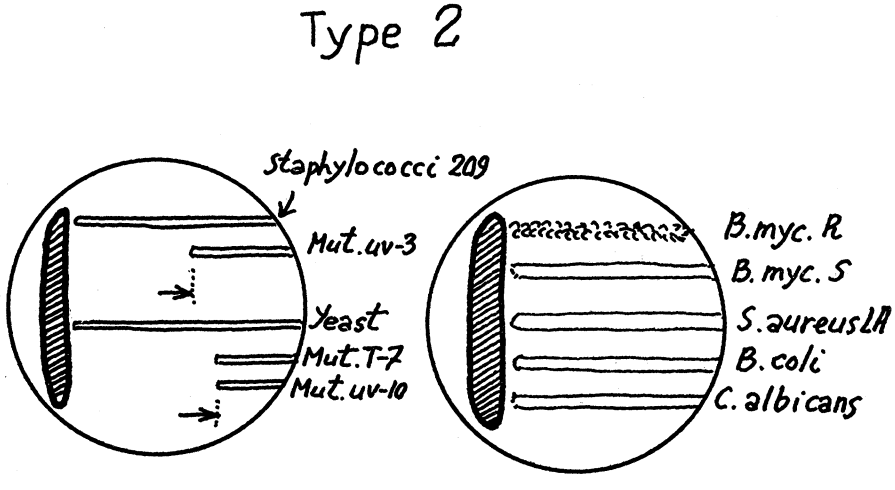

Fig. 2. Selective inhibitory action of a culture of actinomycete upon mutant strains of staphylococci and of yeast cells with impaired oxidation.

Table 2. Respiration in biochemical mutants of *Staphylococcus aureus* 209 with impaired oxidation (from Gause, Kochetkova and Vladimirova, *9*). Q_{O_2} shows the consumption of oxygen in cubic millimeters per hour calculated for 1 milligram of the dry weight of bacteria.

Culture	Number of experiments	Q_{O_2}
First series		
Control (parent)	10	95.2
Ultraviolet-1	4	65.0
Ultraviolet-2	8	49.5
Ultraviolet-3	4	44.0
Second series		
Control (parent)	10	104.5
Ultraviolet-1	6	64.5
Ultraviolet-2	6	51.7
Ultraviolet-3	6	41.3

normal staphylococci belonging to different strains, nor did they inhibit the growth of the other test microorganisms employed, as is shown in Fig. 1. Therefore, in the usual screening program, these 53 cultures would not be recognized as antagonists and probably would be discarded. Nevertheless, these cultures produce specific antibiotic substances that selectively inhibit the growth of staphylococci with deficient respiration. In this way, the use of biochemical mutants of staphylococci helps us to detect antibiotics which remain "invisible" if the usual method of investigation is employed.

The 53 cultures mentioned above belonged mostly to type 1, which is represented in Fig. 1 These cultures selectively inhibited the growth of only mutant strains of staphylococci with impaired respiration; they were not active against normal staphylococci, yeast mutants, or the other microorganisms employed. Ten of these cultures produced chemical substances which not only inhibited the growth of biochemical mutants of staphylococci but also inhibited in vitro the cells of ascites tumors of mice.

The antibiotic action of the second type, which is shown in Fig. 2, was encountered more rarely. In this case, antagonists inhibited the growth of mutants with deficient respiration in staphylococci as well as in yeasts, whereas the growth of the parent cultures and of the other microorganisms was not affected. We observed only eight antagonists of this type; among these, six cultures inhibited in vitro the cells of ascites tumors of mice.

Some cultures of actinomycetes which selectively inhibit, in petri dishes, the growth of biochemical mutants of microorganisms with deficient respiration, possess also the capacity to produce selectively inhibiting substances in the course of their submerged growth in liquid nutritive media. The isolation of the active substances and the study of their chemical nature are of great interest.

From cultures of various actinomycetes, M. G. Brazhnikova, T. A. Uspenskaia, and I. N. Kovsharova (*11*) isolated chemical substances that possessed selective inhibitory action upon the biochemical mutants of staphylococci with deficient respiration. One of these, antibiotic No. 992, was obtained in crystalline form. This substance is inactive toward normal staphylococci and other microorganisms, but it inhibits the growth of biochemical mutants of staphylococci with deficient respiration (strain ultraviolet-3) in the dilution of 1/5,000,000. That is, the mechanism of action of this antibiotic is directly opposite to that of albomycin, an antibiotic which inhibits only the growth of normal staphylococci and does not affect mutants with deficient respiration (*9*). According to the data of V. A. Shorin and O. K. Rossolimo (*12*), antibiotic No. 992 slightly inhibits in vivo the growth of experimental ascites tumors of mice.

Conclusion

It is too early to say at present whether the chemical substances that selectively inhibit the growth of biochemical mutants of microorganisms might acquire some practical importance in the future for the treatment of malignant growth. But it is already clear that these substances are very interesting from the theoretical viewpoint. The biochemical mutants give us an opportunity to select substances with definite mechanisms of inhibitory action instead of carrying out a purely empirical search for new antibiotics.

The deficiency of respiration may depend upon many different enzymes, and it should be subjected to special analysis. It was found, for example, that mutant strains of staphylococci with deficient respiration (ultraviolet-2 and ultraviolet-3) are not identical. In strain ultraviolet-3 the respiration is more strongly injured and is less sensitive to cyanides than it is in strain ultraviolet-2. We observed antibiotics which did not inhibit the growth of common microorganisms and were at the same time active only against mutants of ultraviolet-2, but not against mutants of ultraviolet-3. In this way the biochemical mutants of microorganisms give us an opportunity to detect "invisible" antibiotic substances, selectively inhibiting some specific biochemical systems of the living cell. It seems probable that further work along these lines may stimulate closer cooperation between those conducting screening programs for antibiotics and biochemists; such cooperation might be helpful in the design of screening programs.

In summary, we can say that, in screening for new anticancer antibiotics, the two following generalizations may be helpful. (i) There is a possibility of obtaining the equivalents of cancer cells in microbiology—that is, biochemical mutants of microorganisms with deficient respiration and probably with some other alterations in cell metabolism which are specific for malignant growth. (ii) There is a possibility of using these biochemical mutants for the detection of new substances that selectively inhibit those specific features of cell metabolism which are characteristic of the malignant growth.

References and Notes

1. O. Warburg, *Uber den Stoffwechsel der Tumoren* (Springer, Berlin, 1926); *Science* 124, 269 (1956); *Experientia* 13, 125 (1957).
2. H. Lettré *et al.*, *Naturwisssenschaften* 44, 335 (1957).
3. J. Leslie *et al.*, *Nature* 178, 1179 (1956).
4. J. Huxley, *Biol. Revs. Cambridge Phil. Soc.* 31, 474 (1956).
5. G. F. Gause, G. V. Kochetkova, G. B. Vladimirova, *Doklady Akad. Nauk S.S.S.R.* 117, 1 (1957).
6. B. Ephrussi *et al.*, *Ann. inst. Pasteur.* 76, 351, 510 (1949).
7. R. Bauch, *Naturwissenschaften* 29, 503 (1941).
8. G. J. Roskin, *Microscopiceskaia Technika* (Moscow, 1951).
9. G. F. Gause, G. V. Kochetkova, G. B. Vladimirova, *Doklady Akad. Nauk. S.S.S.R.* 117, 4 (1957).
10. T. P. Preobrazhenskaia and E. S. Kudrina, in press.
11. M. G. Brazhnikova, T. A. Uspenskaia, I. N. Kovsharova, in press.
12. V. A. Shorin and O. K. Rossolimo, in press.