industrial micro-organisms

The contribution of microbiology to the alleviation of disease and its possible role in helping to make up food shortages, especially in the developing countries, were among the topics reviewed at a symposium on the use of radiation and radioisotopes for the genetic improvement of industrial micro-organisms, held at Agency Headquarters a few weeks ago.

Microbial fermentation industries have undergone revolutionary development in recent years — development which has led to the large-scale production of various organic substances having nutritional, medicinal or other desirable characteristics. Some such uses of "industrial micro-organisms" were discussed in an article in the Bulletin (Vol. 12, No. 2) last year.

The 88 participants in the symposium, from 28 countries, four international organizations and the IAEA, heard first an introductory survey by S. I. Alikhanian, of the Institute for Genetics and Selection of Industrial Micro-organisms, Moscow. Alikhanian pointed out that the combination in the programme of the symposium of questions relating to genetics, biochemistry and technology was not accidental. "The organization of any industrial production based on microbial synthesis," he said, "deals with two problems: the first concerns physiological and biochemical investigation. Microbiologists discover new forms of micro-organism and together with biochemists find their new capacities to synthesise specific products. Sometimes these may be well studied forms of micro-organisms that reveal a capacity to synthesise new substances, as it is with penicillin and streptomycin. However most wild micro-organisms appear to display weak synthesising capacity, hence organization of industrial production based on them is unprofitable.

"The second problem concerning organization of microbiological production is determined by selection of these strains, with the purpose of obtaining a sharp increase in the productivity of wild micro-organisms.

"Parallel with the isolation of new, highly-active strains there develop wide studies of the requirements of selectional strains for nutrient substances, screening of precursors, investigations of optimal aeration conditions, temperature conditions for biosynthesis, methods of preparation and growth on plating material and so on.

"In all aspects of these studies directed to the increase of the productivity of micro-organisms genetics plays the most important role. It is quite obvious that success in the selection of micro-organisms depends on the application of genetic methods. Numerous attempts to ignore genetic methods in the selection of micro-organisms have always led to failure."

Alikhanian, who is recognised as a pioneer in this work, pointed out that genetic methods in combination with the use of ionizing radiation (in particular, X-rays) played the greatest role in the first stage in the development of techniques for the selection of micro-organisms. X-rays, a very active mutagen, were first used 25 years ago by M. Demerec in the isolation of the first mutant strain of *Penicillium Chrysogenum,* X-1612, which was as much as three times as productive as the wild strain 1951-B25. Use of such superactive strains enabled microbiological synthesis to become not only profitable for the production of a number of compounds, but competitive with chemical synthesis.

The use of ultra-violet light was the next mutagenic technique to be developed. *P. chrysogenum* was again the first micro-organism treated; the mutant Q-176 was isolated, displaying activity which exceed that of the original strain by a large factor — and was three times that of the first mutant induced by X-irradiation.

Later, gamma-irradiation and fast neutrons were tested and used successfully. The effectiveness of fast neutrons markedly exceeded that of X-rays and UV-light. Chemical mutagens were also used ; when chemical techniques were used in combination with UV-light the mutagenic effect was found to be increased to a greater extent than would have been expected by simple addition. Strains now in use are about 1500 times as productive as the original *P. chrysogenum.*

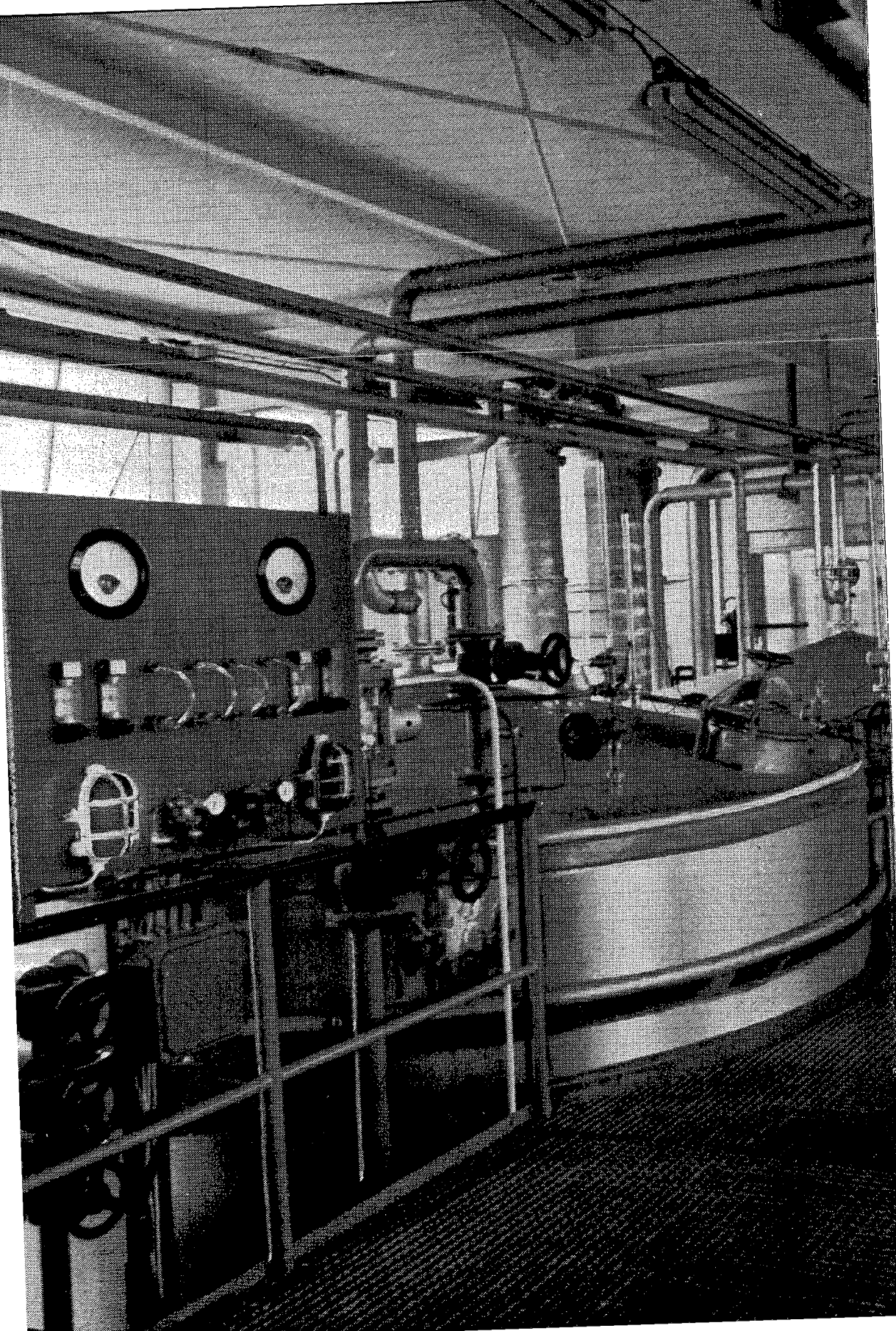
A characteristic feature of this first period in the development of techniques for microbial selection was its empiricism, said Alikhanian. "The thing is, that until recently theoretical problems of experimental mutagenesis developed without connection with practical problems... There was no necessity to employ mutagens in selection, simply because of the absence of microbiological industry. Much later, after active investigations on experimental mutagenesis were initiated in 1943—45 with the development of wide-scale penicillin production and production of other antibiotics, there arose a question of genetic selection work with micro-organisms.

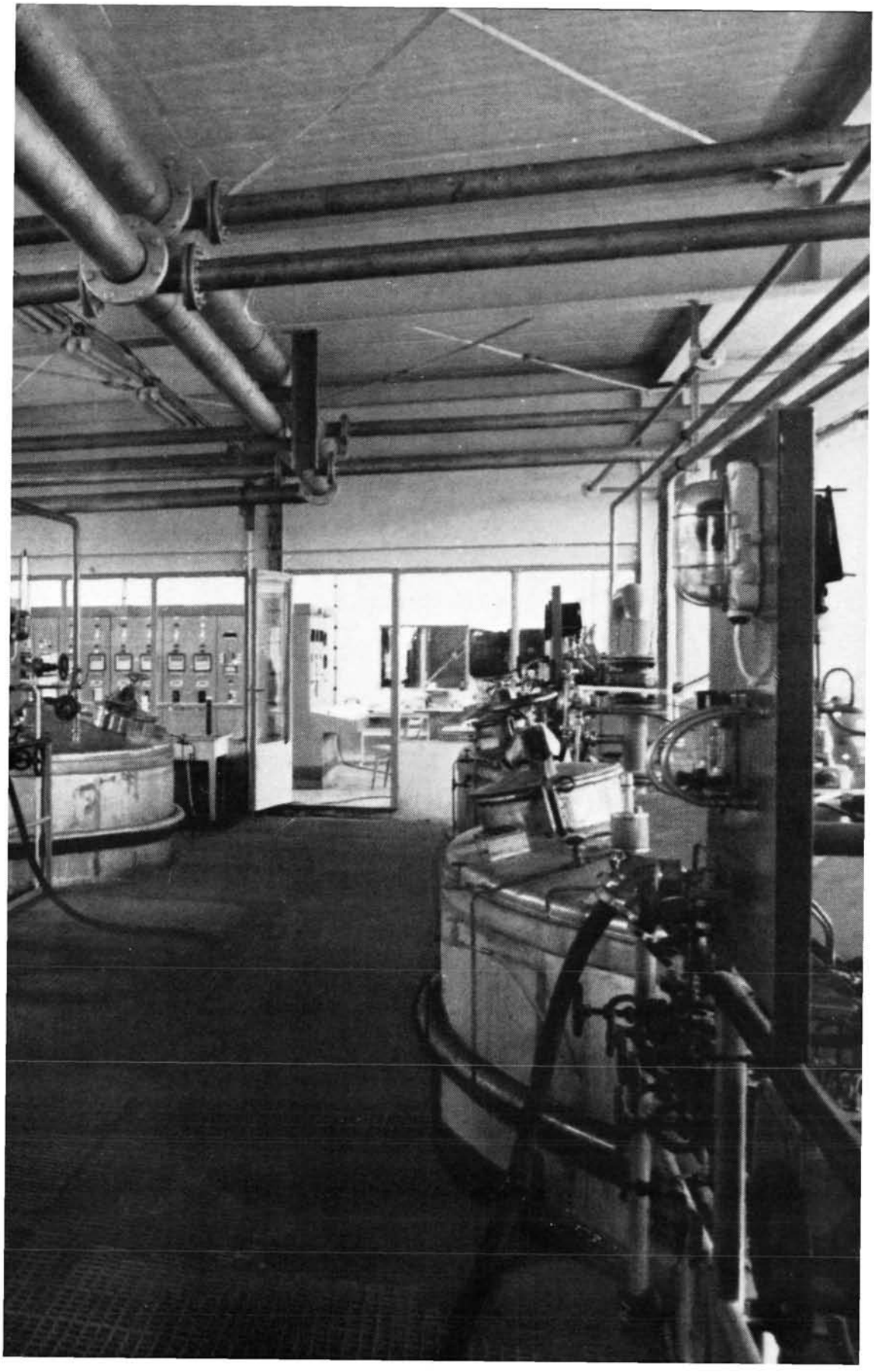
"... The second period in the development of genetic science is coupled with the rapid growth of studies on microbial and molecular genetics, and the development of microbiological industry."

The speaker went on to deal with some problems and some recent work in micro-organism breeding practice, some of which are reviewed here.

Investigations relating to the regulation of constitutive synthesis might prove very important, he said. The level of constitutive enzymatic synthesis did not depend on the composition of the medium, aeration or other environmental conditions. On the other hand, the levels of

Photo next page:
The interior of a yeast fermentation room, showing the central control panel and the scale of the apparatus. Photo: Vogelbusch Gesellschaft m. b. H., Vienna





constitutive synthesis differed for different enzymes. One problem which arose here was in the estimation of the *potential* level for such constitutive synthesis; solution of this problem might have both practical and theoretical significance.

In the same way, use of data obtained from genetic studies of cell regulatory mechanisms led to consideration of the isolation of highly-productive strains of amino acid producers. All such producers appeared to be auxotrophs [1]. Now, after a series of studies of regulating genes, research workers were investigating regulatory mutants — mutants with impaired systems of regulation, which might be selected from cultures resistant to amino acid analogues. It seemed reasonable to consider the possibility of isolating strains which combined auxotrophic mutation and mutation affecting the regulatory mechanism of the cell.

Initial experiments with a lysine producer resistant to s-(2-aminoethyl) cystein-inhibitor of the first common enzyme of amino acid synthesis of the aspartate family were a success. Such an enzyme lost sensitivity to combined inhibition by lysine and threonine, and as a result lysine synthesis increased.

Recently, in some countries studies had been developed in relation to the production of cell protein on hydrocarbons contained in oil, and a number of plants had been built to enable production of such protein on an industrial scale. In most cases the protein was produced as dried biomass of yeasts, but recently, at the Xth International Congress for Microbiology in Mexico, the investigation of methods for the production of protein by micro-organisms which yielded large amounts of substances such as catalase or some other protein with a desired amino acid composition had been suggested.

In all, participants in the symposium heard 30 papers and, during the meeting, visited a bakers' yeast production plant. The rationale for the Agency's interest in this extraordinarily complex subject area was given in a paper presented at the end by R. Mukherjee, scientific secretary for the symposium.

Briefly, it is that "micro-organisms in the fermentation industry give promise of being an economic source for food, nutritional supplements, pharmaceutical products and organic acids that are greatly needed or are lacking in adequate amounts for a large sector of the world's population.

"Great strides have been made in conventional agricultural practices that have led to increased productivity of crops, livestock and the control of pests as well as in the storage and preservation of foods, particularly in the temperate climates," he said. "However, the cost of transporting these products is high even if there were an adequate amount of them to supply the increasing world needs. The evidence is now clear, and has been reinforced through this symposium, that strains of micro-organism when properly fitted in to the technology of the fermentation industries can make a very significant addition to the food, medical and industrial needs of the world.

"Micro-organisms ... can be transported at very low cost, increase at a phenomenal rate when grown on a proper medium and — of great significance — can utilize waste products while producing substances important to the health and welfare of a country. Probably of even greater interest

1) Auxotrophs are organisms which depend on supplementation of nutrient substance(s).

is the fact that it is in the tropical and sub-tropical regions of the world, where food supplements are most greatly needed, that there exist waste products such as molasses, cornsteep liquors and carbohydrate-rich root crops that could be converted to the needs of these countries through use of micro-organisms.

"One worker in a yeast producing plant can on the average produce about 60 tons of dry protein per year, whereas his counterpart in conventional agriculture can produce only 6 tons of dry protein per year. It would be expected that this difference in productivity may be increased even further as fermentation technology is improved and new strains of micro-organisms are developed."

The Agency's programme of radiation biology, said Mukherjee, was directed toward encouragement of the use of ionizing radiation for the improvement of micro-organisms that produced beneficial products. But its interest did not end there: it was concerned with the total effort leading to the economic production of antibiotics, amino acids, organic acids, vitamins, hormones and so on, and in attempting to increase the rate of information exchange in this part of microbiology.

To this end the Agency had initiated a coordinated research programme in radiation microbiology, designed to impart "much needed information, guidance, and encouragement for the participants from institutes of the developing countries." At present nine scientists from eight countries — including five developing countries — were taking part in this programme; their research activities included studies on mutation induction and selection of improved strains, development of genetic systems by various processes of recombination, biochemical and genetic analysis of the biosynthetic regulatory processes, and the development of strains suitable to utilize locally-available carbon-rich substrates as nutrient.

Other organizations which were represented at this meeting were Foratom (the Forum Atomique Européen), UNESCO, UNIDO and WHO. It is expected that the proceedings will be published in a few months.