were made available ? The advocacy of this development is strongly supported; indeed a call is made for a higher priority in the pursuit of this subject. It is believed, too, that industry would be bound to respond to its solution for manifold reasons. Taking the long view, as regards the future of the coal fields, this could well be an almost universal method for the utilization of coal, for it is the only foreseeable solution at present of the sulphur problem.

R. J. SARJANT

BIOCHEMISTRY OF HUMAN TUMOURS

DURING November 17–19, an international conference on the biochemistry of human tumours was held at the University of Wisconsin, Madison. This meeting was sponsored by the Biochemistry Committee of the Cancer Chemotherapy National Service Center, National Cancer Institute, U.S. Public Health Service, with the present writer serving as programme chairman. Those invited to attend included five scientists from Europe and fifty-five from the United States. Among these were pathologists, clinical cancer chemotherapists, biochemists and biologists. The need for this conference arose from the conviction of members of the Biochemistry Committee, expressed in a guest editorial (James F. Holland and Charles Heidelberger, Human Cancer, the Primary Target", *Cancer Research*, 20, 975–6; 1960), that despite formidable technical difficulties, increased biochemical research must be carried out on human tumours. This should include studies on the enzyme patterns of human tumours as compared with normal tissues, as well as research on the metabolism and mechanism of action of active tumour-inhibitory drugs in the human cancer patient and *in vitro* with specimens obtained at surgery or by biopsy.

The meeting was opened by James F. Holland (Buffalo, U.S.A.), who pointed out some of our major deficiencies in knowledge about human cancer. He directed attention to the variability of human tumours, even of the same kinds, with respect to growth-rates and patterns, and to their response to chemotherapeutic agents. Even though extensive work has been done on biochemical mechanisms of action of various antimetabolites in susceptible and resistant transplanted rodent tumours, similar work on human tumours is almost totally lacking. Moreover, there is little information on enzyme activity in human tumours, and on the effects of tumours on host metabolism, as exemplified by terminal cachexia. Holland felt there is a particular need to develop new methods to measure the growth-rates of inaccessible tumours and to get a measure of the amount of stromal and necrotic contamination of tumour specimens.

A brief review of human tumour pathology was given by Paul Kotin (Los Angeles), who showed a number of slides to illustrate diversity even among similar types of human tumours. He made the point that there is little, if any, correlation between the histological appearance of tumours and the carcinogenic stimuli that produced them. Therefore, he suggested that biochemical patterns reflecting essential consequences of the carcinogenic process might be studied more meaningfully in pre-neoplastic tissues, such as carcinomas *in situ*, rather than in the fully developed and highly diversified tumours. He also suggested that work be carried out to understand the differences in natural history between certain tumours in children and their counterparts in the adult. The morphological considerations were carried to another order of resolution by Francoise Haguenau (Paris), who discussed the electron microscopy of human tumours. She demonstrated with elegant slides the diversity of human tumours with respect to the appearance of the submicroscopic cellular organelles. Furthermore, some cells that appeared similar in the light microscope were found to differ markedly in their ultramicroscopic morphology, and she was able to distinguish on this basis between the α- and β-cells of Caspersson.

A. R. Curreri (Madison) discussed the surgeon's role in the collection of surgical specimens, and emphasized the willingness of surgeons to collaborate in co-operative enterprises provided that the purpose of the research was made clear. Much discussion followed, which was summarized by Holland: "Know thy surgeon and love thy biochemist".

A detailed discussion of the techniques that have been developed for low-temperature storage, with maintenance of viability, of cells grown in tissue culture was given by Cyril Stulberg (Detroit). The application of these methods to the storage of sections of human tumours, which would be essential in any proposed large-scale co-operative studies of tumour enzymology, remains to be worked out.

Because of the extreme heterogeneity of specimens of human tumours, it is clear that micro- and ultra-micro-methods are required in most cases in order to obtain meaningful information. Accordingly, David Glick (Minneapolis) and Oliver Lowry (St. Louis) discussed in some detail the elegant and precise techniques that they have developed for the quantitative assay of enzymes extracted from samples the size of common histological sections and by microdissection. These involved mainly colorimetric, spectrophotometric, and fluorimetric methods. Another technique for enzyme assays with very small samples was described by V. R. Potter (Madison), involving the use of labelled substrates of high specific activity, paper chromatography, and the use of automatic recording equipment for the direct determination on the paper strip of the radioactivity in the substrate and product.

His extensive work on the amounts of some twenty enzymes in 800 human tumours by various histological staining methods was described by R. G. J. Willighaghen (Leyden). An enormous diversity of results was found; some enzymes were present in some tumours and absent in others from the same organ and of the same histological type. However, in some cases it has been possible to predict the clinical behaviour of tumours based on histochemical observations. Using similar techniques, L. Wattenberg (Minneapolis) reported that aminopeptidase, an enzyme not found in the normal gastric mucosa, occurs in many gastric malignancies, and in this

metabolic feature resembles intestinal metaplasia of the stomach and also the mucosa of the small bowel.

James Danielli (London) pointed out that no single variable has been found in tumours to serve as a basis to design new drugs. Therefore it is necessary to summate small differences in order to obtain responses. He discussed his interesting research on the Walker 256 rat carcinosarcoma with aromatic nitrogen mustards containing a urethan group, which require enzymatic cleavage by the tumour before the mustard becomes active. He was able to demonstrate that feeding a chemotherapeutically inactive phenylurethan derivative to rats raised the level of the cleavage enzyme in the tumour, which resulted in a potentiation in chemotherapeutic effectiveness when the substituted mustard was given afterwards. This approach of inducing an enzyme in tumours that would liberate the active drug from a non-toxic precursor was discussed extensively.

Henry Pitot (Madison) described his work (in collaboration with V. R. Potter) on the enzymatic constitution of a series of transplanted rat hepatomas as compared with that of normal and regenerating liver. A number of marked differences were found, and enzymological evidence was presented to suggest strongly that the Novikoff hepatoma arose from bile-duct cells, whereas the Dunning hepatoma arose from parenchymal liver cells. In the case of the Morris 5123 hepatoma no qualitative difference between the tumour and liver was found in assays of some thirty different enzymes. However, several enzymes in liver that show marked variations in response to substrate administration and dietary changes showed no such response in the 5123 hepatoma under similar conditions. This may indicate that some biochemical control mechanisms may be lacking in this tumour.

A study, by conventional biochemical techniques, of about twelve enzymes and six analytical parameters was reported for twenty-three human tumour specimens by George Boxer (Rahway, New Jersey). He stressed the diversity and heterogeneity of the samples as examined by various criteria and the necessity of standardizing rigorously the enzymatic assays and determining stability under the frozen storage conditions employed in this work. The difficulty of deciding the basis to which the enzyme activity should be referred (wet weight, protein, deoxyribonucleic acid, etc.) was discussed in some detail. He and several others have used an analysis for collagen as an indication of the amount of connective tissue contaminating the tumour samples, but this has not as yet proved satisfactory.

C. de Duve (Louvain) discussed a strategic approach to chemotherapy by classifying the various enzymes that affect or are affected by a given drug. The target enzymes are acted on by the drug : a positive target would be the release or activation of enzymes that might kill cells, such as lysosomes ; a negative target would be an enzyme which if inhibited would result in cell death. Auxiliary enzymes were defined as those acting directly on the drug ; a positive auxiliary would convert the drug into a more active form ; a negative auxiliary would detoxify the drug. It was agreed that this would be a useful way to consider mechanisms of drug action. de Duve also believed that it would be more useful to study a few enzymes very completely in human tumours than to carry out a superficial survey of the amounts of many enzymes.

At this point there followed thirty-two brief presentations by the remainder of the participants in the conference. These ranged from the purely conceptual to the presentation of potentially useful techniques, and stressed work on the mechanism of action of various chemotherapeutic agents in human beings. An open discussion followed.

The meeting was marked by a congenial spirit established among the participating pathologists, surgeons, clinicians, and biochemists that augured well for the future of this type of research, which requires close co-operation and mutual understanding among workers. The pall cast over the conference by the pathologists' demonstration of the tremendous heterogeneity and diversity of human tumours was never completely dispelled, however, and the most urgent problem to be solved before productive work can be done is to develop quantitative methods to cope with this situation, so that meaningful criteria can be established. It was agreed that all work should be done under strict histological control. The majority of the participants felt, with some vigorous dissenters, that this work would have to be carried out on a small scale in order to produce meaningful results. Some expressed the view that overall metabolic studies in cellular systems, particularly when dealing with mechanisms of drug action, might be more useful than enzyme assays, but all agreed that more work with human tumours was needed.

On the day following the conference, a small meeting of some of the participants was held to discuss organization of research in this field. It was agreed that informal study groups would be set up among the participants of the conference to discuss, by correspondence and afterwards by informal meetings, three general areas considered to be of crucial importance at this time. The first will deal with the problem of the diversity and heterogeneity of human tumours and the development of suitable techniques and a basis for meaningful biochemical studies. The second will involve asking biochemists interested in the enzymology of tumours to make recommendations as to which enzymes would yield most fruitful results on investigation. The third will deal with the stimulation of additional research on mechanisms of drug action in human cancer patients and *in vitro* in surgical and biopsy specimens of human tumours. Initially these activities will be co-ordinated and sponsored by the Cancer Chemotherapy National Services Center. Investigators who wish to have additional information or wish to participate in this programme are invited to communicate with Dr. J. Leiter or Dr. S. Schepartz at that agency.

The challenge of research with human tumours is one that is worthy of the interest, ingenuity, and participation by all scientists interested in cancer, working as individuals or as interdisciplinary teams. In order for this challenge to be met adequately a mobilization of scientific talent all over the world is required. The meeting recently held in Madison was a modest step in this direction. It is to be hoped that similar meetings may be held in other regions of the world, encouraged perhaps by some of the participants in this meeting. At some time in the near future, once some of the formidable technical problems have been solved, it may be appropriate for some agency such as Unesco to arrange for the exchange of information in this field at an international level.

Charles Heidelberger