THE BIOCHEMISTRY OF ABNORMALITIES IN CELL DIVISION

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Carbohydrates have been considered for a long time to be the fuel of the tissues of the body, but it is only during the last few years that some of the mechanisms whereby the energy from carbohydrate catabolism is utilized have been revealed. MEYERHOF has done more than any other biochemist to show how carbohydrate metabolism involves phosphorylation and how the phosphorylated products can yield energy for other biological processes. A remarkable property of living machinery is that it can make, repair and maintain its own working parts. Cancer tissue has a high carbohydrate metabolism and a high rate of cell division. The carbohydrate metabolism, partly aerobic and partly anaerobic, yields the energy necessary for cell division and the maintenance of the nuclei which seem to control the processes of cell division. The main constituents of cell nuclei of both normal and cancer cells appear to be proteins and nucleic acids, and the carbohydrate metabolism is possibly merely concerned with production of high energy phosphate bonds which will yield energy in a form available for synthesis of nucleic acids and possibly of proteins. Inhibition of these processes will stop cell division and so inhibit growth. If the inhibition is such that cell division is impeded but not stopped then the incidence of abnormalities such as damaged chromosomes, mutations or cancer might be increased.

Normal cells are not capable of continuous growth. If they continue to receive surplus nourishment after attaining a certain limiting size they divide. If the process of cell division is inhibited, then growth is also inhibited. In the cell division or mitosis in which nuclei and plasmagenes play a dominant rôle there is exact partitioning of the chromosome material between the daughter cells. The occurrence of spontaneous chromosome abnormalities and mutations shows that chromosomes are not absolutely stable. The induction of changes or mutations by physical and chemical agents indicates that the nuclear material is sensitive and vulnerable to conditions of the environment.

Perhaps the most sensitive indication of abnormalities of cell division is the occurrence of mutations, as these are functional manifestations of such abnormalities. If the change of normal cells to cancer cells is a somatic mutation then the fact that an agent is carcinogenic is an indication that it is mutagenic. Actually most of the mutagenic agents which lend themselves to testing have been found to be carcinogenic and many carcinogenic agents have been shown to induce mutations.

Many of the means which will induce cancer and increase the mutation rate of animals will inhibit the growth of animals or of tumours growing in animals. Such inhibition of growth by carcinogenic hydrocarbons was described by HADDOW[1]. Inhibition of growth in this way may form the basis for therapy of cancer.

The more complete correlation between the actions we are considering was first

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shown with X-rays. Radiotherapy of cancer started (GRUBBE[2]) soon after Röntgen's discovery of X-rays. Seven years later FRIEBEN[3] reported that a skin cancer had developed in a man who had been exposed to X-rays. MULLER[4] showed that X-rays increased the incidence of mutations in Drosophila and PAINTER AND MULLER[5] and KOLLER[6] found that X-radiation caused visible abnormalities in chromosomes.

All these effects can be produced by certain chemical agents, such as the nitrogen mustards and urethane, which for this reason have been called radiomimetic. The carcinogenic hydrocarbons such as 1:2:5:6-dibenzanthracene are also radiomimetic agents. Table I shows the grouping of the different effects.

TABLE I

REFERENCES TO EFFECTS PRODUCED BY X-RAYS AND BY CHEMICAL COMPOUNDS WITH RADIOMIMETIC ACTIONS

	Treatment of Cancer or Inhibition of growth	Induction of Cancer	Induction of Mutations	Chromosome Damage
X-rays	GRUBBE[2]	FRIEBEN[3]	MULLER[4]	PAINTER AND MULLER[5]
Nitrogen Mustard	RHOADS[7]	BOYLAND AND HORNING[8]	AUERBACH, ROBSON AND CARR[9]	BOYLAND, CLEGG KOLLER, RHODEN AND WARWICK[10]
Urethane	PATERSON, APTHOMAS, HADDOW, AND WATKINSON[11]	NETTLESHIP AND HENSHAW[12]	OEHLKERS[13]	BOYLAND AND KOLLER[14]
1:2:5:6-Dibenzanthracene	HADDOW, SCOTT, AND SCOTT[15]	KENNAWAY[16]	CARR[17]	KOLLER[18]
Methylcholanthrene	HADDOW, SCOTT, AND SCOTT[15]	COOK AND HASLEWOOD[19]	STRONG[20]	—
N:N-di (2-chloroethyl)-2-naphthylamine	HADDOW, KON, AND ROSS[21]	HADDOW, HORNING, AND KOLLER[22]	—	KOLLER[18]
4-Dimethylaminostilbene	HADDOW, HARRIS, KON, AND ROE[23]	HADDOW, HARRIS, KON, AND ROE[23]	—	KOLLER[18]

Another effect which many of these agents produce is the bleaching or greying of hair. This was described in mice exposed to X-rays by HANCE AND MURPHY[24]. A similar effect occurs with nitrogen mustard derivatives either aliphatic (BOYLAND *et al.*[10]) or aromatic (HADDOW *et al.*[22]). This greying of hair is a permanent effect, remaining with the mouse for the remainder of its life. It may be perhaps considered as a somatic mutation and in this respect is analogous to an induced tumour. The change from coloured to white hair which is induced is unlikely to be due to selective survival of more resistant white hair follicles as the skin of the black (C57) or agouti (CBA) mice used do not appear to contain white hairs. This change of colour in a part of the body is a discontinuous variation in properties like the change of normal into cancer cells. Both changes are brought about by the same agents which also induce germinal mutations.

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These agents also cause visible damage to chromosomes and it is probable that the inherited variations are due to change of plasmagenes or to chromosome damage which might not have been visible if the affected cell had been examined. The dose of mutagenic agent which is required to produce visible abnormalities will cause death in many of the treated cells and the new forms arise in the cells which have received a sublethal dose.

The tumours which arise as the result of treatment of cells with a mutagenic agent are possibly derived from a normal host cell which has produced daughter cells differing from the parent cell because of some accidental error or abnormality of cell division. When the total number of cell divisions in the whole mammalian body is taken into account these abnormalities are very infrequent. The chance of their occurrence seems to be made much more probable by the presence of a carcinogenic or mutagenic agent.

If the changes brought about by carcinogenic agents are random variations of the original cells as suggested it is perhaps surprising that different tumours are so similar in their morphology and biochemistry. Each tumour has its own specific characters but the differences between tumours induced by carcinogenic agents are relatively small. Different tumours resemble each other more closely than they resemble the tissue of their origin. Thus tumours have less of the specific functions of the cell from which the tumour arose and tumours have the property of producing lactic acid aerobically. Of the mutations which occur in somatic cells probably many are unable to survive; many will die normally and others will be unable to withstand the attacks of defence processes of the host. Of the numerous mutations which occur only those which produce cells able to survive, grow, and induce the host to provide a blood supply, will become detectable cancers, and for these biological processes specific characters of function and morphology may be required. As the changes are induced by substances which damage the chromosome material (either directly or indirectly) and probably the genes, the chang esare probably the result of loss or inactivation of genes, as it seems unlikely that a toxic agent should add something to the nuclear material. Such changes would be analogous to the mutations induced in Neurospora which result in the loss of ability to carry out some specific chemical process.

The biochemical mechanism which operates when radiations, nitrogen mustards or carcinogenic hydrocarbons induce mutations or cancer, is still obscure. The nitrogen mustards or chloroethylamines are chemically reactive and combine with many tissue constituents and inactivate many enzymes, but particularly the phosphokinases and the pyruvic oxidase enzyme system. In order to produce the chromosome damage and inhibition of the growth of tumours in animals the aliphatic chlorethylamines must have two chloroethyl groups (BOYLAND *et al.*[10]) and the necessity of two reactive or polar groups for chemotherapeutic action against cancer was suggested earlier (BOYLAND[25]). GOLDACRE, LOVELESS, AND ROSS[26] have suggested that the two active groups join chromosome parts by cross linkage of protein or other constituents. As a result of these additional cross linkages the division of chromosomes is hindered and breakages and damage to the chromosomes occurs. This theory would not account for the action of urethane (which seems to have no chemically reactive groups) and it is difficult to see how arsenicals such as sodium arsenite could act in this way. Sulphydryl compounds are the only known tissue constituents with which arsenite is known to react. As there is very little cysteine or other sulphydryl compound in chromosomes (DAVIDSON AND LAWRIE[27]) combination of chromosome chains by union of sulphydryl groups through an arsenic atom is unlikely to occur. It also seems improbable that X-rays would cause stable cross

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linkages between chromosome parts to be formed. The current theory of the action of radiations on cells is that they oxidize sulphydryl groups through the production of peroxide or other oxidizing agent within the cells. They could therefore unite peptide chains by conversion of sulphydryl groups to the disulphide forms. The low concentration of cysteine in the chromosomes which was suggested as a difficulty in the theory as applied to the action of arsenicals would also apply to X-rays. A linkage through arsenic might, however, be more stable than a disulphide link which would probably be reduced in processes of cell metabolism. This hypothesis of cross linkage within chromosomes being the cause of abnormalities may be of value in investigating the action of drugs on tumour cells, but it is possibly of no more value than the knowledge that in the chloroethylamine series and other compounds two active groups are required for the biological actions considered.

The hypothesis which the author put forward (BOYLAND[28]) postulates that the effects of these substances are due to inhibition of enzymes, particularly the phosphokinases or enzymes involving oxidative phosphorylations necessary for production and metabolism of the nucleic acid required for the maintenance of normal chromosomes and genes. Since then BARRON, DICKMAN, AND SINGER[29] have shown that phosphoglyceraldehyde dehydrogenase is particularly sensitive to the action of X-rays, and MEYERHOF AND WILSON[30] have described the inhibition of hexokinase and phosphohexokinase with phenyl urethane.

Investigations carried out during the war showed that two enzyme systems were particularly sensitive to the poisoning action of vesicants. Of the phosphokinases, hexokinase was first shown by DIXON AND NEEDHAM[31] to be inhibited by low concentrations of mustard gas and nitrogen mustard. Later CORI and his co-workers[32] found that phosphokinases in general are inhibited by vesicants. PETERS, SINCLAIR, AND THOMPSON[33] found that the arsenical vesicant, lewisite and other vesicants inhibit the pyruvic oxidase system. The known phosphate transferring enzymes are concerned with the building up of energy rich phosphate bonds in phosphoric anhydrides and acylphosphates. Enzymes of this type must be concerned in the biosynthesis of the nucleotides and nucleic acids. Although we know very little of the specific phosphokinases involved in nucleic acid synthesis, the fact that all known phosphokinases are easily inhibited by sulphur mustard and nitrogen mustards would suggest that nucleic acid synthesis should be inhibited by these substances. The synthesis of proteins may also involve phosphorylation of the terminal carboxyl group of a peptide chain and reaction of the resulting acyl phosphate with a fresh amino acid molecule to give a new peptide link and liberate phosphate. A model for this reaction is the formation of glutamine from phosphoryl glutamic acid and ammonia (SPECK[34], ELLIOT[35]). The enzymes concerned with nucleic acid and protein synthesis need investigation and for this the mitotic poisons may be useful tools.

The substances which induce mitotic abnormalities differ greatly in their apparent chemical reactivity. The aliphatic nitrogen mustards are very reactive substances, the aromatic chloroethylamines react slowly, but the aromatic carcinogenic hydrocarbons are rather inert. The French theoretical chemists DAUDEL, PULLMAN and their associates (DAUDEL[36]) have shown that the carcinogenic hydrocarbons have regions, known as the K regions, in which there is high electron density, which in the majority of the carcinogenic hydrocarbons includes an activated phenanthrene double bond. The activation is enhanced by substituents such as benzene rings or methyl groups (which repel elec-

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trons) in such positions that they increase the electron density of the phenanthrene double bond. This double bond in the more potent carcinogenic hydrocarbons such as 9:10-dimethylbenzanthracene has a chemical reactivity for some addition reactions approaching that of an aliphatic ethylenic bond and even greater than that of the ethylene bond of some stilbenes. This theory which is now substantiated by experimental evidence, suggests that the more active carcinogens in any particular series of aromatic compounds are those which are on the whole the more chemically reactive.

Phenanthrene itself reacts readily with osmic acid (CRIEGEE, MARCHAND, AND WANNOWIUS[37]) and the carcinogenic hydrocarbons react even more rapidly (BADGER[38]). Osmic acid adds on to the double bond of the K region to form an adduct, which can be easily hydrolysed to give *cis*-dihydroxydihydro-derivatives.

Perbenzoic acid is another reagent which appears to react with carcinogenic hydrocarbons at rates varying with the carcinogenic activity. This reagent was shown to react with 20-methylcholanthrene and 3.4-benzpyrene more rapidly than with anthracene and phenanthrene (ECKHARDT[39]) before the theory of the K region of carcinogens had been developed. In looking for a means of measuring the relative reactivity of the K region, the reaction of perbenzoic acid with a series of carcinogens has been determined. Some of the data obtained are shown in Table II. The figures show that the carcinogenic hydrocarbons react at about the same rate as the carcinogenic aminostilbenes. This suggests that the bond of the K region of the hydrocarbons is as reactive as the ethylenic bond of the stilbene molecule and as the azo group of the carcinogenic dimethylaminoazobenzene.

The fact that dimethylaminoazobenzene dosed to animals in which it induces hepatoma is found in a combined form in the protein of the liver (MILLER AND MILLER[40]),

TABLE II

REACTION OF CARCINOGENS AND RELATED SUBSTANCES WITH PERBENZOIC ACID

M/50 solutions of substances dissolved in carbon tetrachloride with M/50 perbenzoic acid at 25° C. The remaining perbenzoic acid was estimated iodometrically and the results are expressed as millimols of perbenzoic acid used per mol substrate.

Compound	Time in hours				Carcinogenic activity
	3	24	48	72	
9:10-Anthraquinone	5	0	0	5	—
Naphthalene	0	0	20	10	—
Phenanthrene	5	15	20	25	—
9:10-Phenanthraquinone	0	0	25	35	—
Anthracene	5	35	60	80	—
1:2-Benzanthracene	0	25	70	95	—
1:2:5:6-Dibenzanthracene	0	15	35	95	+
5-Methyl-1:2-benzanthracene	0	40	40	110	+
4-Aminostilbene	32	85	120	140	+
2-Acetylaminofluorene	0	40	100	160	+
3:4-Benzpyrene	5	90	130	202	+
3:4-Benzphenanthrene	45	90	145	220	+
Stilbene	5	20	110	295	?
3:4:5:6-Dibenzcarbazole	95	215	265	322	+
20-Methylcholanthrene	105	275	340	405	+
2'-Methyl-4-dimethyl-aminostilbene	215	405	465	535	+
2'-Chlor-4-dimethyl-aminostilbene	175	390	500	590	+
Dimethylaminoazobenzene	455	590	615	—	+

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shows that a carcinogen can react with tissue protein. As the hydrocarbons react with perbenzoic acid almost as rapidly as dimethylaminobenzene and the azo group of the latter compound is expected on theoretical grounds to have an electron density of the same order as the carcinogenic hydrocarbons, the carcinogenic hydrocarbons might also be expected to combine with some tissue protein in a similar way.

Although the French theoretical chemists have concentrated on the K region of a particular carcinogenic hydrocarbon it is perhaps worth noticing that these substances have two active regions. Many carcinogens such as 1:2:5:6-dibenzanthracene and 3:4-benzphenanthrene contain two active phenanthrene double bonds or K regions. In those carcinogenic hydrocarbons with only a single K region the groups which activate that region may also increase the activity of a second part of the molecule. Thus, in the potent carcinogen 9:10-dimethyl-1:2-benzanthracene, the two methyl groups not only make the 3:4 bond more active than in the unsubstituted 1:2-benzanthracene but also increase the chemical reactivity of the 9:10 or meso positions. Such meso substituted anthracene derivatives are extremely susceptible to many chemical reactions, such as photo-oxidation. The metabolism of carcinogens also shows that another region of the molecule (the benzene ring adjoining the K region) is liable to attack *in vivo*. Although it is quite clear that carcinogenic hydrocarbons must have one centre of high chemical reactivity, they also have a second active centre, either a second phenanthrene double bond, active meso positions, or an amino group as in the aminostilbenes or the amino-azobenzene derivatives.

The reactivity of hydrocarbons is also shown by metabolism experiments with non-carcinogenic hydrocarbons such as naphthalene (BOOTH AND BOYLAND[41]); (YOUNG[42]) and anthracene (BOYLAND AND LEVI[43]) as well as with the carcinogenic hydrocarbon 3:4-benzpyrene (WEIGERT AND MOTTRAM[44]). These hydrocarbons undergo the reaction of perhydroxylation involving the addition of the elements of hydrogen peroxide with formation of dihydroxydihydro derivatives or diols. In the case of the non-carcinogenic hydrocarbons the addition of the hydroxyl groups occurs at the centres with highest electron density. But in the carcinogenic hydrocarbons which have been examined the oxidation occurs in positions in a ring adjacent to the K region — not in the reactive K region itself. This may be because the more reactive carcinogens combine with some tissue constituent through the double bond so that only regions of secondary activity are available for the oxidative process. The investigation of 3:4-benzpyrene metabolism showed that the dihydroxydihydro-benzpyrene formed by metabolism in isolated skin was combined to some tissue constituent. The combination, however, could be destroyed by treatment with wet acetone. Studies with 1:2:5:6-dibenzanthracene containing radioactive carbon (HEIDELBERGER AND JONES[45]) have shown that a small part of the carcinogen remains in animals for many months after injection. Thus there are several indications, that the carcinogenic hydrocarbons can react with some, as yet unidentified, tissue constituents.

Although these hydrocarbons have some of the biological effects of nitrogen mustards they do not appear to inhibit the hexokinase of tumours; the anaerobic glycolysis and respiration of tumours is the same whether they are growing normally or are inhibited by 1:2:5:6-dibenzanthracene (BOYLAND AND BOYLAND[46]). On the other hand inhibition of tumour growth by nitrogen mustard is accompanied by a decrease in the anaerobic glycolysis of the tissue (BOYLAND *et al.*[10]). This inhibition of tumour growth by carcinogens, such as 4-dimethylaminostilbene or 1:2:5:6-dibenzanthracene, is only

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seen if the treated animals are maintained on a low protein diet (ELSON AND HADDOW[47]). This finding indicates that the inhibition of growth is probably due to interference with protein metabolism which can be overcome if the protein intake of the host is sufficiently high. As 1:2:5:6-dibenzanthracene causes abnormalities of chromosomes these experiments suggest that chromosomes require an adequate supply of amino-acids for their proper maintenance.

The rates of diffusion and reaction are probably important characteristics of the nuclear poisons which have been discussed. The compounds must, presumably, react in or near the nucleus to produce their effects. For this they must diffuse through the cell to the nucleus more rapidly than they react with the constituents of the tissue through which they are passing, unless they have a specific affinity for the particular constituents concerned with nuclear behaviour. The aliphatic nitrogen mustards react very rapidly in the body, having a life of only a few minutes, but they do not react instantaneously with any reagent and diffuse rapidly so that some unchanged molecules may reach the nucleus.

The evidence put forward supports the theory that chemical carcinogenic and therapeutic agents for cancer combine with tissue constituents and that physical agents cause some chemical change in chromosome constituents. GOLDACRE, LOVELESS, AND ROSS[26] suggest that it is the chromosomes themselves which are affected while the author considers that the effects are due to inhibition of enzymes concerned in metabolic processes involved in maintenance and functioning of the chromosomes.

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SUMMARY

1. The association of the effects of chromosome damage, induction of mutations and induction of cancer with a number of agents is discussed.

2. Examination of the reaction of a series of carcinogenic compounds with perbenzoic acid shows that carcinogenic hydrocarbons react more rapidly than simpler non-carcinogenic hydrocarbons and at about the same rate as nitrogenous aromatic carcinogens.

3. The suggestion that the nitrogen mustards and possibly other carcinogens produce their effects by inhibition of enzymes necessary for normal functioning of cell nuclei is considered.

RÉSUMÉ

1. La relation entre les lésions des chromosomes, l'induction de mutations et l'induction du cancer par un nombre d'agents est discutée.

2. L'examen de la réaction d'une série de composés cancérigènes avec l'acide perbenzoïque démontre que les hydrocarbures cancérigènes réagissent plus rapidement que les hydrocarbures non-cancérigènes et à la même vitesse à peu près que les substances cancérigènes azotées aromatiques.

3. La suggestion que les moutardes azotées et peut-être d'autres substances cancérigènes produisent leurs effets en inhibant les enzymes nécessaires pour le fonctionnement normal du noyau cellulaire est considérée.

ZUSAMMENFASSUNG

1. Das Verhältnis zwischen Chromosomenverletzung, Hervorrufen von Mutationen und Krebsbildung durch verschiedene Agentien wird diskutiert.

2. Die Untersuchung der Reaktionen einer Reihe von cancerogenen Verbindungen mit Per-

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benzoesäure zeigt, dass die cancerogenen Kohlenwasserstoffe schneller reagieren als einfachere, nicht cancerogene Kohlenwasserstoffe und ungefähr ebenso schnell wie stickstoffhaltige aromatische Krebsstoffe.

3. Der Verfasser schlägt vor, dass die Chloräthylamine und möglicherweise auch andere cancerogene Substanzen ihre Wirkung durch Hemmung der für die normale Funktion der Zellkerne notwendigen Enzyme ausüben könnten.

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