the alteration occurring during heating is referred to as 'heat denaturation' irrespective of whether the heated preparation was previously irradiated or not.

The ability of uv-radiation to decrease the stability of some proteins, particularly of some enzymes, may be responsible for the sensitization to heat of some living organisms by exposure to sub-lethal doses of the radiation, such as has been observed with *Paramecium* (Bovie & Klein, 1919; Giese & Crossman, 1946), bacteria (Curran & Evans, 1938) and yeast (Anderson & Duggar, 1939). The same phenomenon was also described with X-ray irradiated chick embryos (Strangeways & Fell, 1928). In contrast to isolated proteins, living organisms can recover from the effect of irradiation if sufficient time is allowed between exposure to the radiation and to heat.

SUMMARY

1. When proteins having specific activities, such as enzymes or viruses, are exposed to ultraviolet radiation, the specific activity need not to be lost as a result of the first of a series of alterations. Chymotrypsin molecules can be altered by the radiation so that their stability at temperatures around 37° at pH 7 is decreased although they are still proteolytically active. The increased rate at which tobacco mosaic virus is denatured on heating after irradiation results from a series of changes following a step which is associated with the loss of specific activity (infectivity).

2. Quantum yields for inactivation of chymotrypsin and tobacco mosaic virus computed from the data obtained in this work agree with those obtained by previous workers.

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The Purine and Pyrimidine Composition of some Deoxyribonucleic Acids from Tumours

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Since Vischer and Chargaff elaborated procedures for the chromatographic separation and analysis of the purines and pyrimidines, which require only milligram quantities of nucleic acid (e.g. Vischer & Chargaff, 1948; Chargaff, Vischer, Doniger, Green & Misani, 1949), a fairly large number of specimens of deoxyribonucleic acid from a variety of sources have been analysed in different laboratories. Such studies are associated chiefly with the American workers Chargaff and his collaborators (Chargaff, 1950, 1951), with Mirsky and his colleagues (cf. Daly, Allfrey & Mirsky, 1950), and with Marshak (cf.

Marshak & Vogel, 1950; Marshak, 1951). In this country Markham & Smith (1949) and Wyatt (1951*b*) have also made valuable contributions to technique and analytical results.

Most of the figures show variations in the purine and pyrimidine contents of the deoxyribonucleic acid specimens from different biological sources, and Chargaff (1950) concluded that his evidence indicates that, while the deoxypentose nucleic acids (DNA) extracted from cells of different organs of one species are very similar in composition, significant differences occur in the DNA from different

species. He first drew attention to the remarkable differences in the purine and pyrimidine composition between the DNA of mammalian tissues and that of certain bacteria (Vischer, Zamenhof & Chargaff, 1949).

Very few analyses of the purine and pyrimidine content of 'tumour' DNA have so far been reported. Chargaff (1950) examined a specimen from metastatic carcinoma in human liver, Laland, Overend & Webb (1952) analysed a specimen prepared in this laboratory from mouse-sarcoma tissue, and Elmes, Smith & White (1952) recorded figures for a specimen obtained from human leukaemic spleen. Recently, Asimov & Simon (1953) investigated a series of tumour nucleic acid samples but reported only the adenine and guanine values. The composition of a number of animal and human tumour specimens, determined by methods now accepted as giving dependable results, is recorded in this paper.

MATERIALS AND METHODS

The source and general designation of the tumours is given in Table 1. The mouse strains are designated according to the system devised by the Committee on Standardized Nomenclature for Inbred Strains of Mice (1952). Figures in parentheses, column 2, Table 1, and column 1, Table 3, refer to the number of specimens isolated and analysed. The animal tumours included spontaneous (mouse-mammary), transplanted (sarcoma and mammary), and chemically induced (rat-liver) types. In the case of mouse tumours, pooled specimens of non-necrotic tissue were used. The human cancers were obtained after surgical removal and had not been subjected to radiation. They were selected as typical malignant growths from which it was possible to dissect sufficient neoplastic material free from necrosis, and with negligible normal tissue. A number of specimens from normal tissues have also been analysed by the same techniques. The methods employed conformed to those described and carefully checked by Chargaff (1950), Mirsky & Pollister (1946) and Wyatt (1951*a, b*) and, therefore, need only be outlined briefly as follows: The minced tissues were extracted several times with 0.14*M*-NaCl and the deoxyribonucleoprotein then removed by extraction with *m*-NaCl. This was precipitated as fibrous nucleoprotein in dilute NaCl solution, redissolved and deproteinized by shaking with octanol:chloroform. After separation from the

protein gel in the centrifuge, the solution of nucleic acid was further treated repeatedly with this mixture to remove protein. The purified DNA was finally precipitated with 80% (v/v) ethanol, dissolved in distilled water, dialysed for 24 hr. and dried *in vacuo* after freezing at -60° . In some cases specimens were compared before and after treatment with *N*-NaOH at 37° , which Wyatt (1951*b*) found to remove traces of pentose nucleic acid effectively and without loss of purines or pyrimidines.

The purines and pyrimidines were liberated by hydrolysis at 100° for 1 hr. with 72% (w/v) HClO_4 (Marshak & Vogel, 1951) using 0.5 ml. for 50 mg. and afterwards adding 1.5 ml. of distilled water. 20 μl . of this hydrolysate were used for each chromatogram. The separation of the bases was effected by the descending filter-paper strip method in glass tanks using isopropanol (60 ml.) + 6*N*-HCl (35 ml.) + propylene glycol (5 ml.). This satisfactorily separated all the bases (adenine, guanine, cytosine, and thymine), and also uracil and 5-methyl cytosine when present, after the solvent front had moved about 35 cm. in some 36 hr.

The positions of the separated 'spots' were detected by the usual photographic technique. The bases were eluted from the specified areas of the dried filter paper with 4 ml. 0.1*N*-HCl and the optical densities of the solutions were determined at the appropriate ultraviolet wavelengths using 0.1*N*-HCl extracts of similar sized, adjacent areas of the filter paper as blank controls.

The nitrogen and phosphorus contents of the specimens were also determined, the former by micro-Kjeldahl estimation and the latter by Holman's (1943) colorimetric method. Also, the deoxyribose values were compared by the diphenylamine colour reaction of Dische (1930), using a purified calf-thymus preparation as standard, the colour value of this being designated 100 units. The values are given in Table 1.

RESULTS

Since it has been convincingly shown that the molar ratios of the nucleotides in DNA are not equal, and because even the exact stoichiometric relationship of phosphorus to total base is not beyond question, there appeared to be no advantage for the present study in expressing results in terms of moles of base/equiv. of phosphorus, or in calculating the molar quantities to a total of 4.0. Accordingly, all values have been expressed on a molar basis relative to adenine = 1.00.

Table 1. *Composition of tumour nucleic acids*

Specimen	Source	Tumour	P (%)	N (%)	Dische value
1	C3H-strain mouse (2)	Spontaneous breast adenocarcinoma	7.8	12.7	93
2	C3H-strain mouse (2)	Transplanted breast adenocarcinoma	7.9	12.7	93
3	IF-strain mouse (1)	Breast tumour: 5th transplant	8.1	13.2	96
4	IF-strain mouse (2)	Breast tumour: 32nd transplant	8.3	13.7	99
5	Stock mouse (3)	Transplanted 'spindle-celled' sarcoma	8.3	13.6	98
6	Stock rat (2)	Transplanted 'spindle-celled' sarcoma	8.66	14.6	100
7	Stock rat (1)	Transplanted 'Walker' tumour	7.4	11.6	89
8	Stock rat (1)	Liver tumour: 2-acetyl-aminofluorene feeding	7.1	12.9	84
9	Human (1)	Carcinoma of breast	8.0	13.1	92
10	Human (1)	Carcinoma of uterus	7.8	12.6	90
		Calf thymus (control)	8.54	14.3	100

Table 2. *Molar ratios of bases in DNA from animal and human tumours relative to adenine = 1.00*

Source of specimen	G	C	T	A/G	T/C
1	0.82	0.76	0.98	1.22	1.29
2	0.79	0.83	1.20	1.27	1.44
3	0.80	0.84	0.98	1.25	1.17
4	0.77	0.80	1.00	1.30	1.25
5	0.68	0.75	0.87	1.47	1.16
6	0.89	0.75	0.99	1.12	1.32
7	0.70	0.69	1.04	1.44	1.51
8	0.76	0.70	1.01	1.32	1.44
9	0.69	0.71	1.10	1.45	1.54
10	0.78	0.72	0.92	1.28	1.28
Leukaemic spleen (Elmes <i>et al.</i> 1952)	0.75	0.68	0.82	1.33	1.20
(Liver metastasis) Sigmoid colon carcinoma (Chargaff, 1950)	0.67	0.56	1.00	1.49	1.79

Specimens numbered as in Table 1. Adenine = 1.00. A, adenine; G, guanine; C, cytosine; T, thymine.

Table 3. *Molar ratios of bases in DNA from normal tissues relative to adenine = 1.00*

Source	G	C	T	A/G	T/C
Calf thymus nuclei (3)	0.85	0.71	0.99	1.01	1.40
Rat liver (2)	0.86	0.73	0.93	1.16	1.27
Human spleen (2)	0.88	0.75	0.93	1.14	1.24
Human foetal thymus (1)	0.89	0.77	0.95	1.12	1.23
Wheat germ (1)	0.90	0.76	0.92	1.11	1.21

The relative purine and pyrimidine contents of the tumour nucleic acid specimens are given in Table 2. Usually, three separate hydrolysates were made and three chromatograms run for each hydrolysate. The greatest value for the standard error of the mean for such independent analyses was ± 0.02 . In Table 3 are shown the mean results obtained with a number of representative specimens from normal tissues.

DISCUSSION

Relative to adenine = 1.00, all the guanine values in our tumour-DNA are between 0.68 and 0.89, and those for cytosine 0.69–0.83. The thymine content in all specimens is of the same order as the adenine, ranging from 0.87 to 1.20, and the ratios adenine/guanine (A/G) lie between 1.22 and 1.47, and the thymine/cytosine (T/C) ratios between 1.17 and 1.54. Thus the general pattern of these tumour-derived nucleic acids is essentially that of the DNA from non-cancered animal tissue, as distinct from that of some bacterial DNA's in which the guanine value, relative to adenine = 1.00, has been found to be 2.31, the cytosine 2.1, and the thymine molarity about 0.85 (Laland *et al.* 1952; Vischer *et al.* 1949).

In some instances, however, individual values diverge from those of normal tissues more than would be expected from consideration of the experimental errors, for example, the low value of guanine and thymine in the mouse-sarcoma nucleic acid, the relatively high figure for guanine in the rat-sarcoma

nucleic acid, and the high value for thymine in that from the transplanted breast tumour of the C3H-mouse.

The significance of such data must be considered in light of the practical difficulties which are encountered in selecting and procuring sufficient and suitable amounts of analogous, normal tissue for comparison. Also, it should be appreciated that both normal tissues and tumours contain varying proportions of several cell types, though usually the cancered tissue contains a much higher proportion of one type. Such variation in the cellular composition of the tissues may to some extent account for the appreciable differences in the values reported by different workers for the DNA from normal organs of the same species. Other factors must, however, be involved since, to cite one example in which the question of cell mixture does not arise, there is a difference of 20% between the thymine value of Chargaff (1950) and that of Elmes *et al.* (1952) for human sperm DNA.

The range of thymine values in our analyses was greater even than this, which indicates that in certain instances tumour nucleic acid composition may vary from that of the normal tissue from which the neoplasm is 'derived'. In this connexion it may be of some significance that the mouse sarcomas contain a very high proportion of cells of the 'spindle' type; these develop rapidly, and histological sections invariably show a good proportion of cancer cells in mitotic division. These character-

istics might combine to produce the optimum conditions under which the different composition of the DNA's could be detected chemically.

It is not possible from the available figures to discern any consistent difference from the normal-cell DNA composition which is characteristic of cancer-cell DNA. The results, however, suggest that it would be profitable to study a more comprehensive series of specimens from non-proliferating cells of normal tissues, and from proliferating and neoplastic cells.

SUMMARY

1. Specimens of deoxypentose nucleic acids (DNA) have been prepared by standard procedures from spontaneous and experimentally induced animal tumours and some from human cancers. The purine and pyrimidine composition was determined by hydrolysis with perchloric acid, chromatographic separation on filter paper, and measurement by ultraviolet spectroscopy.

2. The ratios of adenine to guanine and thymine to cytosine was greater than 1.0 in all specimens, and in this respect the tumour-DNA resembled that from non-cancerous animal tissues. The most significant deviation was the low thymine value of mouse-sarcoma DNA.

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