[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WASHINGTON UNIVERSITY]

A Method for the Extraction and Assay of Nucleic Acid Fragments in Tissues[1]

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A method is described for the extraction, separation, identification and assay of various nucleotides, nucleosides, purines and pyrimidines occurring in tissues in a loosely bound or uncombined state. The method employs the techniques of paper chromatography and absorption spectrophotometry, and uses dimethylformamide as the solvent for tissue extraction. The method has been applied to an investigation of the nucleic acid fragments present in nine normal and four cancerous Swiss mouse tissues, and the results are tabulated. Data are shown for tissue samples extracted immediately after removal of the tissues from the animal, and for tissue samples extracted after an autolysis period of four hours at room temperature.

Record[2] may be found of numerous instances in which nucleotides, nucleosides, purines and pyrimidines have been isolated from tissues by methods which would suggest that the isolated products did not arise from the degradation of polymeric materials during the isolation procedure, but to our knowledge there has been no study in which the occurrence of such compounds in an uncombined state has been systematically investigated. Since nucleic acid metabolism and growth are so intimately related, a knowledge of the occurrence of nucleic acid fragments in a variety of tissues might be of considerable value. We have therefore undertaken to develop a method for the assay of nucleic acid fragments in tissues.

A brief outline of the method employed is as follows (see Experimental for complete details): an appropriate sample of the tissue to be investigated is homogenized for 20 minutes at 0° with an excess of N,N-dimethylformamide (DMF), a neutral, relatively inert solvent capable of dissolving a great variety of substances. The DMF solution is separated from the tissue residue by centrifugation, and evaporated to dryness under vacuum. The solid material remaining from the evaporation contains nucleoside polyphosphates, nucleotides, nucleosides, purines, pyrimidines, amino acids, certain pyridine compounds, fatty material and unknown compounds. After a purification step to remove fatty material, the extracted compounds are separated by the technique of two-dimensional paper chromatography, using three developing solvents, however, in place of the usual two. The individual spots on the paper chromatogram are identified by reference to the behavior of known substances when chromatographed in an identical way, and by a comparison of the ultraviolet absorption spectra of the eluates of the spots in acidic and basic media with the corresponding spectra of known compounds. The optical density of an eluate at an appropriate wave length and certain other information permits the calculation of the amount of the compound extracted.

Utilizing the methods briefly outlined above, a number of tissues were examined under two sets of conditions. Under the first set of conditions, the tissues were removed from the animal as quickly as possible and extracted immediately in the hope of obtaining values representative of the tissue in the living animal; under the second set of conditions, the tissues were allowed to age for a period of four hours at room temperature before extraction in order to show the effect of autolysis. In Table I are summarized data pertaining to the occurrence of 24 nucleic acid fragments and related compounds in nine normal Swiss mouse tissues; in Table II are summarized similar data for four cancerous Swiss mouse tissues.

It should be emphasized that the values cited in Tables I and II represent amounts of material extracted under the conditions of the extraction procedure given in the experimental section; they do not necessarily represent the total amount present in the tissue in an essentially "free" state. This may be illustrated by an experiment in which 100 μg. of cytidine was added to a mixture of DMF and 2 g. of homogenized fresh liver. Only about 15% of the cytidine was recovered. Since enzymatic reactions do not occur in the DMF mixture (see Extraction of Tissues under Experimental), it would seem most unlikely that the loss of cytidine could be ascribed to a chemical conversion to other materials, and since in several other tissue extractions amounts of cytidine very much in excess of 50 μg./g. of wet tissue have been isolated, the loss of cytidine cannot be due to the insolubility of cytidine in the solvents used in the extraction process. The only conclusion, then, is that the finely divided liver is able in some manner to take cytidine from the DMF solution. Similar experiments with other added nucleic acid fragments gave similar results. These experiments show that the extent to which a particular compound may be extracted depends on the manner in which it is partitioned between DMF and the tissue homogenate. Further substantiation of this conclusion is given by the fact that if a tissue residue from the DMF extraction procedure is re-extracted, smaller but still significant amounts of those compounds which appeared in large amounts in the first extract may be isolated. Those compounds which appeared in small amounts in the first extract did not appear in the second extract. Since one of our primary aims was to demonstrate occurrence of the various nucleic acid fragments, we did not routinely re extract the tissue residues a second time, for if a compound did not appear in the first extract, it was unlikely that a second or even third extraction would provide enough material for detection. As a result, the total content of a "free" nucleic acid fragment in a particular tissue may be considerably higher than the value cited in Table I or Table II. The term "free" nucleic acid fragment should be qualified; as

(1) This work was supported by a grant from the Charles F. Kettering Foundation.

(2) See, for example, S. F. Kerr, *J. Biol. Chem.*, **132**, 147 (1940); S. F. Kerr and K. Seraidarian, *ibid.*, **159**, 637 (1945).

TABLE I

NUCLEIC ACID FRAGMENTS EXTRACTED FROM NORMAL MOUSE TISSUES (μG./G. OF WET TISSUE)[a]

Compound	Heart F[b]	Heart A[c]	Kidney F	Kidney A	Liver adult F	Liver adult A	Liver young F	Liver young A	Liver old F	Liver old A	Lung F	Lung A	Muscle F	Muscle A	Pancreas F	Pancreas A	Spleen F	Spleen A
Adenine	0.5	0	0.3	0	0.9	0	0.2	0.5	0.5	2	0.3	0	0	0	0	0	0.6	0
Guanine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypoxanthine	4	440	25	230	2	280	14	450	2	490	10	6	2	240	12	260	26	200
Uric acid	0	0	0.9	0[d]	0	0	0	0	0	0	0	44	0	3	0	0	0	0
Xanthine	0	190	11	130	0.9	140	+	92	0.8	180	0	10	0	52	0	240	4	570
Cytosine	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	22
Thymine	0	0	0	0	0	0	0	t	0	0	0	0	0	0	0	0	0	0
Uracil	0.9	42	8	210	0.4	84	3	150	0.8	200	0.9	110	0.5	13	7	360	10	220
Adenosine	24	0	35	0	3	0	+	0	1	0	6	0	0	0	0	0	0	0
Cytidine	0	37	0	0	0	0	0	0	0	0	0	120	0	7	7	1350	7	580
Guanosine	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Inosine	14	61	93	37	9	23	21	32	2	25	17	6	5	440	6	190	27	58
Thymidine	0	0	0	0	0	0	0	t	0	0	0	0	0	0	0	0	0	61
Uridine	3	1	29	37	8	250	8	340	2	380	2	7	0	21	11	770	11	170
Xanthosine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0
Adenylic acid	170	0	470	20	51	0	23	0	58	7	130	4	47	0	100	270	170	19
Cytidylic acid	5	0	0	0	0	0	0	0	0	0	0	0	0	0	85	140	0	0
Guanylic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	t	0
Inosinic acid	16	0	58	t	5	20	0	2	0	0	9	0	36	300	0	0	4	0
Thymidylic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0
Uridylic acid-I	0	0	35	0	21	0	0	0	15	0	8	0	2	0	0	0	3	0
Uridylic acid-II	0	0	100	0	75	13	22	0	27	0	16	0	0	0	130	120	40	4
Adenosine triphosphate-adenosine diphosphate-	22	0	100	0	14	0	18	0	25	0	21	0	123	0			0	0
Diphosphopyridine nucleotide	0	0	28	0	6	0	8	0	0	0	14	0	0	0		0	0	0

[a] Where a numerical value is not given, a blank space denotes a compound whose presence or absence could not be definitely established, the symbol t denotes possible presence in amounts less than 0.5 μg./g. of wet tissue, and the symbol + indicates presence in an amount of the order 10 μg./g. of wet tissue. [b] The symbol F refers to "fresh" samples. [c] The symbol A refers to "aged" samples. [d] A value of 33 was found in a duplicate experiment.

used here it indicates a fragment not bound in nucleic acids or other compounds, but it is not meant to exclude fragments held in less tightly bonded combinations such as enzyme–substrate complexes.

There are other limitations on the data in Tables I and II. For example, a tissue sample of 2–5 g. is made up of organs from several mice. There undoubtedly are variations for a given organ depending on the state of health, nutrition, etc., of the mouse from which the organ was removed. By using several organs to make up a tissue sample, a sort of averaging is obtained, but the values do vary from one sample to another for a particular type of tissue. Actual duplicate runs show these variations to be small; nevertheless, it is necessary to point out, that the results cited in the tables under the headings "fresh" and "aged" for a given tissue are not completely comparable since a different tissue sample was employed for each type of experiment. It should also be noted that the experiments on aged tissues were designed solely as exploratory experiments, and that no attempt was made to control variables in the aging process other than temperature and time. Thus, the cancerous tissues, which had to be sliced up considerably in order to remove necrotic material, were perhaps better able to obtain oxygen indirectly required for oxidative enzymes such as xanthine oxidase than tissues like liver and heart which were allowed to age as intact organs, and which may have been aging under essentially anaerobic conditions.

Brief comment should be made concerning some specific compounds: guanine was not isolated from any mouse tissue although guanine added to a mixture of finely divided fresh liver and DMF may be isolated, indicating that the absence of guanine is not due to a difficulty in the extraction procedure. Due perhaps to the fact that the homogenization procedure employed may not have broken up cell nuclei, fragments related to deoxyribonucleic acid were not extensively extracted. All nucleosides tabulated appear to be ribose derivatives as indicated by chromatographic behavior and periodate titration[3] data. In numerous fresh tissues two separate and distinct spots whose eluates exhibited uridylic acid absorption spectra were found. One of the two spots corresponded in position to known uridine monophosphate, and was tabulated as uridylic II. The other spot appeared immediately above uridylic II, and was tabulated as uridylic I. Because it moved a lesser distance in the two organic solvent mixtures employed in the first direction of the chromatographic separation, uridylic I is probably a uridine polyphosphate[4] compound. (Compare the movements of adenosine mono- and polyphosphate compounds in Fig. 1.) Since the molecular weight and extinction coefficient of the

(3) J. S. Dixon and D. Lipkin, to be published.

(4) See S. H. Lipton, S. A. Morell, A. Frieden and R. M. Bock, THIS JOURNAL, **75**, 5449 (1953), and references therein.

TABLE II

NUCLEIC ACID FRAGMENTS EXTRACTED FROM MALIGNANT MOUSE TISSUES (μG./G. OF WET TISSUE)[a]

Compound	Hepatoma F[b]	Hepatoma A[c]	Mammary gland carcinoma F	Mammary gland carcinoma A	Rhabdomyosarcoma F	Rhabdomyosarcoma A	Tumor No. 3 F	Tumor No. 3 A
Adenine	0.2	0	0.2	0.3	0.3	0.4	0	0
Guanine	0	0	0	0	0	0	0	0
Hypoxanthine	12	130	9	58	9	340	22	23
Uric acid	0.5	64	2	36	0	8	0	63
Xanthine	10	120	2	51	t	150	3	110
Cytosine	0	0	0	0	0	0	0	0
Thymine	t	+	0	t	t	7	0	12
Uracil	14	210	2	200	10	590	11	260
Adenosine	0.4	0	3	0	6	0	5	0
Cytidine	0	0	0	3	0	0	1	0
Guanosine	0	0	2	0	0.9	15	0	0
Inosine	6	12	21	15	18	33	50	2
Thymidine	0.8	+	0	t	t	t	0	0
Uridine	7	88	14	160	6	54	10	15
Xanthosine	0	3	0	0	0	0	0	0
Adenylic acid	20	10	80	0	190	1	32	1
Cytidylic acid	0	0	0	0	4	0	0	0
Guanylic acid	0	0	0	0	0	0	0	0
Inosinic acid	0	0	2	0	6	t	0	0
Thymidylic acid	0	0	0	0	0	0	0	0
Uridylic acid-I	10	1	0.9	0	19	0	11	0
Uridylic acid-II	11		27	0	33	t	15	0
Adenosine triphosphate–adenosine diphosphate	3	0	102	0	24	0	46	0
Diphosphopyridine nucleotide	0	0	16	0	42	0	34	0

[a,b,c] Same as in Table I.

presumed polyphosphate compound were unknown, uridylic I was tabulated in terms of an equivalent quantity of uridine monophosphate. We have not as yet carried out any experiments to establish the position of phosphate in the sugar moiety of the extracted nucleotides.

Some of the findings shown in the tables suggest that considerable information concerning nucleic acid metabolism could be obtained by the further application of these experimental procedures. For example, a possible explanation of the large increase in uracil and uridine in aged tissues is that the action of ribonuclease has degraded nucleic acid, giving uridylic acid which in turn is degraded further. Such enzymatic action,[5] however, should lead to an increase in cytidylic acid or its degradation products as well. In pancreas and spleen this is the case, for cytidine is found in amounts comparable to the amount of uracil and uridine liberated by autolysis. In liver and the cancerous tissues, no cytidine is observed. The answer may be that cytidine or cytidylic acid are rapidly deaminated in these tissues, and thus appear in the extract in the form of uracil or uridine. Such a hypothesis could be tested easily by allowing liver or a cancerous tissue to age in the presence of added cytidine or cytidylic acid, and to compare the amounts of uracil and uridine obtained in such an experiment with the corresponding values obtained from a control experiment without added substrate. Numerous experiments of this type suggest themselves; we are presently conducting some of them and shall await their results before speculating on interpretations of the data in Table I and II.

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Experimental

Handling of Tissues.—Tissues are removed from test animals as quickly as possible, taking care to cut away all visible necrotic material from cancerous tissues, and unwanted appendages from normal organs. If it is convenient, the tissue may be extracted at once; if not, the tissue may be frozen in liquid nitrogen and extracted at a later time without altering the results of the extraction. All results for "fresh" samples reported in this article were obtained on tissues which had been frozen 30 to 60 seconds after removal from the animal, and extracted after 2 to 18 hours of standing in liquid nitrogen. Tissues used in the "aged" experiments were removed from liquid nitrogen, thawed, and allowed to stand for 4 hours at room temperature in an open vessel before extraction. All tissues used in this work were Swiss mouse tissues; all the cancerous tissues were transplanted tumors with the exception of mammary gland carcinoma, a spontaneous tumor.

Extraction of Tissues.—1.0 to 5.0 g. of tissue is added to 25 ml. of N,N-dimethylformamide (DMF) contained in a Bailey–Walker extraction flask (Corning Cat. No. 5180) cooled by an ice-bath to 0°. A set of small cutting blades inserted in the extraction flask and rotated at high speed accomplishes the homogenization of the tissue. (The action is similar to that of a "Waring Blendor.") The time of homogenization is set arbitrarily at 20 minutes. (Actually, experiments have been run which show that homogenizations for periods of 10 minutes to one hour give quite comparable results. This result shows, incidentally, that enzymatic action is inhibited by the presence of DMF since extensive degradations occur in tissues allowed to stand by themselves for one hour.) The mixture is transferred to a centrifuge tube, centrifuged, the supernatant removed, the residue stirred with an additional 15 ml. of DMF, and again centrifuged. The two supernatants are combined and evaporated to dryness at room temperature under vacuum in a rotating evaporator.[6] The DMF is removed without bumping in about one hour; the residue is left under vacuum another hour at about 0.01 mm. pressure.

The residue is now washed in the evaporator flask with two 10-ml. aliquots of sodium-dried benzene to remove fatty material. The residue remaining in the evaporator flask and a small amount of residue obtained from the centrifugation of the benzene extract are taken up in 1 *N* NH_4OH solution (0.6 ml. per g. of tissue), and the solution centrifuged at 3400 r.p.m. (about 2000 *g*/g.). The supernatant from this centrifugation (hereafter referred to as the ammonium hydroxide fraction) contains a portion of the nitrogen bases and nucleosides, as well as most of the nucleotide and other phosphate-containing materials. It is generally not a clear solution, but contains finely divided suspended matter.

The benzene extract from above contains, aside from fatty material, additional amounts of the nitrogen bases and nucleosides, as well as a small amount of nucleotide

(5) G. Schmidt, *et al.*, *J. Biol. Chem.*, **170**, 739 (1947); C. E. Carter and W. E. Cohn, THIS JOURNAL, **72**, 2604 (1950).

(6) We used a device similar in principle to that described by S. M. Partridge, *J. Sci. Instr.*, **28**, 28 (1951), but considerably altered in design.

material. It is treated further as follows[7]: the benzene extract is centrifuged and the supernatant decanted. The residue is air-dried and combined with the ammonium hydroxide fraction as described above. The benzene supernatant is evaporated in the vacuum evaporator to a volume

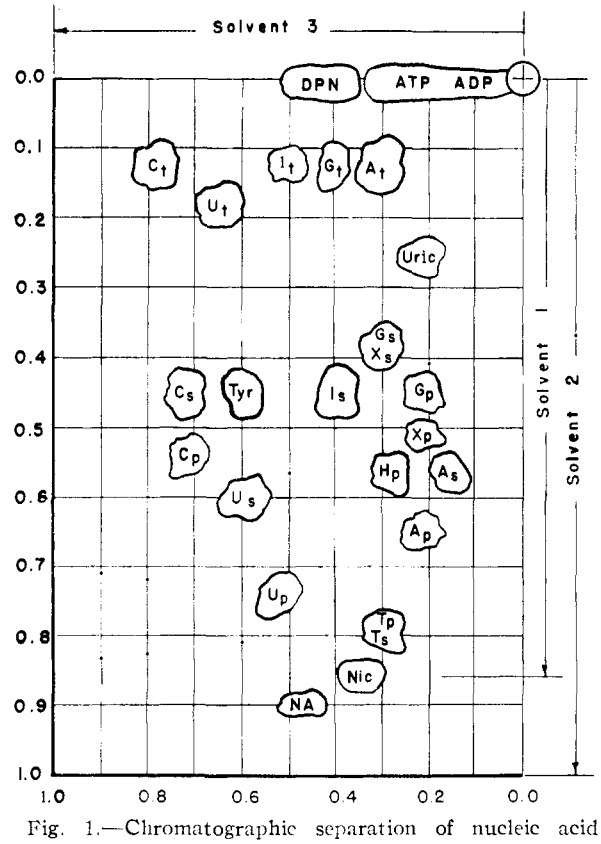

Fig. 1.—Chromatographic separation of nucleic acid fragments. Symbols are as follows: the subscripts t, s and p refer to nucleotides, nucleosides and purine or pyrimidine bases, respectively. Thus, At is adenylic acid; As, adenosine; Ap, adenine; Ct, cytidylic acid; Cs, cytidine; Cp, cytosine; Gt, guanylic acid; Gs, guanosine; Gp, guanine; Hp, hypoxanthine; It, inosinic acid; Is, inosine; Ut, uridylic acid; Us, uridine; Up, uracil; Ts, thymidine; Tp, thymine; Xp, xanthine; Xs, xanthosine. Other compounds shown are NA, nicotinic acid; Nic, nicotinamide; Tyr, tyrosine; DPN, diphosphopyridine nucleotide; ATP, adenosine triphosphate; ADP, adenosine diphosphate; Uric, uric acid. The ordinate represents a combined R_f scale for solvents 1 and 2 with respect to the farthest advance of solvent 2; the abscissa an R_f scale for solvent 3. The composition of the solvents is given in the Experimental section.

(7) ADDED IN PROOF.—A technique for removing fatty material which is simpler and makes unnecessary the separate treatment of a benzene fraction is as follows: the original residue obtained from the evaporation of the DMF solution is dissolved in 1 *N* NH_4OH, using 15 ml. of 1 *N* NH_4OH/g. of tissue extracted. This solution is shaken with $CHCl_3$ (1 ml. of $CHCl_3$/3 ml. of 1 *N* NH_4OH), and the mixture centrifuged. The result is an NH_4OH layer, a clear $CHCl_3$ layer, and a white, gummy interfacial layer. An aliquot of the NH_4OH layer is removed, and the extraction process with $CHCl_3$ repeated two more times. An aliquot of the NH_4OH layer from the final extraction is then removed, evaporated to dryness, and dissolved in 10 ml. of 1 *N* NH_4OH/g. of tissue, an aliquot of which is used for paper chromatography. This final 1*N* NH_4OH solution is perfectly clear and contains essentially all the nucleic acid fragment material as revealed by the fact that the $CHCl_3$ solutions and the white, gummy material (dissolved in ethyl alcohol) show no appreciable absorption in the neighborhood of 2600 Å. characteristic of the nucleic acid fragments

of about 3 ml. This solution is then placed on a 5 inch strip 8 cm. from the top of a 7″ × 22″ piece of Whatman No. 1 filter paper. No attempt is made to confine the solution to the 5 inch line, rather the solution is spread out about 1.5″ on each side of the line to prevent trapping of the nucleic acid fragments in the fatty material. The paper is hung in a closed chromatography box, and washed in the descending direction with benzene. The fats are washed down the paper with the benzene; the nucleic acid fragments are left near the origin (see below for method of locating). The area of paper containing the nucleic acid fragments is removed from the paper, cut into small pieces and allowed to stand overnight with 20 ml. of 1 *N* NH_4OH. The mixture is centrifuged, the NH_4OH solution decanted, and the paper residue washed with two more 20-ml. aliquots of 1 *N* NH_4OH. The combined eluates are taken to dryness in the vacuum evaporator, and the residue taken up in 0.25 to 0.5 ml. of 1 *N* NH_4OH.

The final NH_4OH solution from the benzene fraction may be combined with the original NH_4OH fraction, or the two solutions may be chromatographed separately. We adopted the latter course in the experiments reported here. In working with the NH_4OH fraction, it was our practice to chromatograph aliquots corresponding to 0.25 to 2.0 g. of tissue; with the benzene fraction, aliquots corresponding to 2.0 to 5.0 g. of tissue were chromatographed. Amounts in the upper ranges of these limits were employed for "fresh" tissue samples, in the lower ranges for "aged" tissue samples.

Chromatographic Procedures.—The solution to be chromatographed is evaporated on a spot located 8 cm. from the short side and 10 cm. from the long side of an 18.5″ × 22″ sheet of Whatman No. 1 filter paper. The papers are developed in descending fashion in the following manner: the paper is developed in the long direction with solvent 1, a mixture[8] of *n*-butyl alcohol, acetic acid and water (80:20:20 by volume) to a line 4″ from the bottom. The paper is air-dried and rechromatographed in the same direction with solvent 2, a mixture of acetone, *n*-butyl alcohol and water (80:10:10 by volume), until the solvent reaches a line 1.5″ from the bottom. After air drying again, the paper is turned 90° and developed with solvent 3, a mixture[9] of saturated $(NH_4)_2SO_4$, isopropyl alcohol and water (79:2:19 by volume). The use of two developments in the first direction permits utilization of most of the paper area. We were unsuccessful in finding a single solvent mixture aside from solvent 3 which had a satisfactory spread of R_f values for the compounds in which we were interested. The degree to which this solvent system is able to separate a mixture of known nucleotides, nucleosides, purines and pyrimidines on a two-dimensional paper chromatogram is shown in Fig. 1. Advantage is taken of the ability of these compounds to absorb ultraviolet light to locate their positions on the paper chromatogram[10]; illumination of the paper with a suitable ultraviolet source such as a model V-41 Mineralite reveals the nucleic acid fragments as dark spots against a fluorescent background. Also shown in Fig. 1 are tyrosine, nicotinamide and nicotinic acid, three common tissue constituents which are also revealed by ultraviolet light. With the exception of tyrosine, proline and perhaps a few others, the amino acids (as revealed by the ninhydrin reagent) chromatograph in this system between R_f values 0.8 and 1.0 on the solvent 3 scale and therefore do not interfere with the chromatography of the nucleic acid fragments. All chromatographic developments described above were conducted in a room maintained between 23 and 29°.

Spectral Identification of Nucleic Acid Fragments on Paper Chromatograms.—The individual spot to be identified is cut from the paper and sliced into small pieces about 2 mm. square. The paper cuttings are placed in a sintered glass filter (3 ml. volume, fine porosity), 1.0 ml. of 0.1 *N* HCl added, and the solution sucked through the filter after 15 minutes. Five tenths of a milliliter of this solution is placed in a micro cell (Pyrocell Manufacturing Co., New York), and the absorption spectrum determined in the region 3000 to 2200 Å. on a Beckman model DU spectrophotometer. One-tenth milliliter of 10 *N* NaOH is now added to the cell, and the absorption spectrum redetermined in

(8) L. J. Reed, *J. Biol. Chem.*, **183**, 451 (1950).

(9) R. Markham and J. D. Smith, *Biochem. J.*, **49**, 401 (1951).

(10) E. R. Holiday and E. A. Johnson, *Nature*, **163**, 216 (1949), B. Magasanik, *et al.*, *J. Biol. Chem.*, **186**, 37 (1950).

the range 3000 to 2300 Å. (The large quantity of NaOH is required to counteract the effect of the $(NH_4)_2SO_4$ which is also eluted from the paper; the *p*H of the resulting solution is about 11.) Due to the fact that a small amount of ultraviolet absorbing impurity is extracted from the paper by the eluting agent, these absorption spectra in acidic and basic media must be corrected for the presence of the impurity. A number of experiments on blank papers developed with the chromatographic solvents showed that the correction per square centimeter of paper eluted was sufficiently constant to permit a tabulation of corrections to be applied at various wave lengths. At 2600 Å., for example, the correction in acid solution in optical density units per square centimeter of paper is 0.015, in basic solution 0.028. Finally,

TABLE III

Cpd.[a]	Wave length, Å.	$\epsilon \times 10^{-3}$ 0.1 *M* HCl	Lit. ref. to[b] Abs. curve	ϵ
Ap	2600	13.6	*e*	*e*
Gp	2500	10.0	*e*	*e*
Hp	2500	9.03	*e*	*e*
Uric	2850	11.7	*h*	*h*
Xp	2650	7.93	*e*	*e*
Cp	2750	10.1	*e*	*g*
Tp	2650	7.67	*e*	*e*
Up	2600	8.06	*e*	*e*
As	2600	14.2	*e*	*d*
Cs	2800	13.0	*e*	*e*
Gs	2550	12.2	*e*	*e*, *m*
Is	2470	11.8	*f*	*f*
Ts	2650	10.1	*e*	*e*
Us	2620	9.93	*g*	*g*
Xs	2620	8.90	*c*	*c*
At	2550	15.1	*i*	*f*
Ct	2780	12.7	*g*	*g*
Gt	2550	*m*	*i*	*m*
It	2470	10.7	*i*	*f*
Ut	2620	9.89	*g*	*g*
ATP-ADP[j]	2550	*j*	*i*	*j*
DPN	2600	*k*	*i*	*k*

[a] See Fig. 1 for notation employed. [b] See also F. Schlenck, "Advances in Enzymology," Vol. 9, Interscience Publishers, Inc., New York, N. Y., 1949, pp. 455–535. [c] R. Falconer, J. M. Gulland and L. F. Story, *J. Chem. Soc.*, 1784 (1939). [d] J. M. Gulland and E. R. Holiday, *ibid.*, 765 (1936). [e] R. D. Hotchkiss, *J. Biol. Chem.*, **175**, 315 (1948). [f] H. M. Kalckar, *ibid.*, **167**, 429 (1947). Values of molar extinction coefficients from this article, measured at *p*H 7.2, were adjusted to acid solution. [g] J. McT. Ploeser and H. S. Loring, *ibid.*, **178**, 431 (1949). [h] M. M. Stimson and M. A. Reuter, THIS JOURNAL, **65**, 153 (1943). [i] Determined in this Laboratory. [j] Since ATP-ADP are not separated under the conditions of chromatography used, and since they may not be differentiated spectrally, the chromatographic spot due to these compounds is assayed using an assumed extinction coefficient of 15,000 and an average molecular weight of 468. [k] An extinction coefficient of 18,000 was assumed. [m] Literature values for guanosine and guanylic acid vary considerably (see *b*). We arbitrarily used Hotchkiss' value for guanosine, and assumed guanylic acid to have the same value.

a comparison is made between these corrected absorption spectra and the known absorption spectra of the various nucleic acid fragments in acidic and basic media.

The absorption spectra in acid and base for each of the various purines and pyrimidines are sufficiently unique that identification of these compounds on the basis of compared absorption spectra is unambiguous. The spectra in acid and base for each of the various nucleosides, although different from those of the purines and pyrimidines, are essentially identical with the spectra of the corresponding nucleotides. Thus the identification of the nucleosides and nucleotides cannot be made on the basis of spectra alone. Fortunately, the positions of the two types of substances on the chromatograms are so different that a knowledge of location on the chromatogram taken in conjunction with the spectra permits identification to be made. A number of reference spectra useful for the spectral identification of nucleic acid fragments are tabulated in Table III.

Calculation of Amounts of Nucleic Acid Fragments in DMF Extracts.—The optical density of the 1.0-ml. eluate of a spot in 0.1 *N* HCl solution at an appropriate wave length may be used to calculate the amount of the nucleic acid fragment present by the simply derived equation

$$\text{Amount } (\mu\text{g./g. of wet tissue}) = \frac{(FD - AC)M \times 10^{-3}}{\epsilon w}$$

where

F = dilution factor
D = optical density at the wave length chosen
A = area of the spot in square cm.
C = correction for absorbing impurity per square cm. of paper
M = mol. wt. of the nucleic acid fragment
ϵ = molar extinction coefficient at the wave length chosen
w = wt. of tissue sample represented by the paper chromatogram

The dilution factor, F, is included to take care of those cases where a dilution of the original eluate must be made in order to obtain a reading in a reasonable range of the Beckman spectrophotometer's optical density scale. The areas of the spots were determined with a Keuffel and Esser polar planimeter. The values of ϵ and the wave length chosen are summarized in Table III. The wave lengths chosen correspond closely to characteristic absorption maxima.

The degree of accuracy of this type of analysis is revealed by the experiments tabulated in Table IV in which known quantities of nucleic acid fragments were placed on paper, chromatographed and analyzed.

TABLE IV

Cpd.[a]	μg. of material put on paper	μg. of material recovered from paper	Recovery, %
Hp	26.5	19.0	72
Up	22.4	19.3	86
As	21.6	16.3	75
Cs	17.1	13.1	77
Is	22.4	18.5	82
Us	31.2	21.5	69
At	18.9	20.6	109
Ct	22.1	21.5	97
Ut	24.7	28.9	117

[a] See Fig. 1 for notation employed.

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