Dox medium which contains 1.0 g. NaNO_3 /l. (0.1 %).

As the nitrate concentration is further increased, the growth, indicated by the mycelium yield and the production of sclerotiose, is progressively reduced.

While Fig. 1 indicates that maximum KCl consumption is obtained on media of low nitrate content, Fig. 2 shows a parallelism of sclerotiorine production with growth, in nitrate concentrations up to 0.2 %, and hence independence of the KCl consumption. Above 0.2 % sodium nitrate, it would appear that pigment production is inhibited by the nitrate. As this is also true of KCl utilization, it may be asserted that the production of sclerotiorine is responsive to nitrate concentration only when this factor greatly reduces the amount of KCl metabolized by the organism. The facts justify the previous assertion that pigment production is merely a by-product of the chloride consumption.

SUMMARY

1. The KCl metabolism of *Penicillium sclerotium* is an inverse function of the nitrate concentration of the culture medium, and the pigment yield is unaffected until such time as the chloride consumption is much reduced below normal.

2. The production of sclerotiose is almost inversely proportional to the nitrate content of the medium, yields in excess of 10 % being obtained on a substrate in which the nitrate concentration is half that usually used, as compared with 3 % for the standard (0.2 % NaNO_3) medium.

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Tissue Nucleic Acids

1. RIBONUCLEIC ACIDS AND NUCLEOTIDES IN EMBRYONIC AND ADULT TISSUE

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Desoxyribonucleic acid (thymonucleic acid, chromonucleic acid), known for a long time to be a constituent of the nuclei of animal cells, together with histones and the recently discovered protein chromosomin (Stedman & Stedman, 1943*a*), has been found also in plant tissues (Feulgen & Rossenbeck, 1924), in bean seedlings (Kiesel & Belozerski, 1934; Belozerski, 1936), in onion bulbs (Belozerski, 1939), in the rye grain (Behrens, 1938) and in yeast cells (Delaporte & Roukhlman, 1938). At the same time evidence has accumulated that pentose nucleic acid (ribonucleic acid, yeast nucleic acid, zymonucleic acid, plasmonucleic acid, phytonucleic acid), which differs from desoxyribonucleic acid in containing *d*-ribofuranose in place of *d*-2-desoxyribofuranose and uracil in place of thymine (methyl uracil), and which was originally thought to be solely a plant constituent (cf. Jones, 1920), is present in animal tissues.

Pentose derivatives have long been known to be present in animal tissues (Blumenthal, 1898; Grund, 1902; Wohlge-

muth, 1903; Neuberg, 1904; Mendel & Leavenworth, 1908). As well as the mono- and dinucleotide coenzymes containing adenine, e.g. adenosine triphosphate, the nicotinamide nucleotides and riboflavin-adenine-dinucleotide, pentose nucleotides of other bases have been found, e.g. uridylic acid (Levene & Mandel, 1907) in shell-fish eggs, and all four nucleotides of ribonucleic acid (Calvery, 1928) in hydrolysates of chick embryo pulp. Ribopolynucleotides may also occur. The so-called β -nucleoprotein of the pancreas, originally prepared by O. Hammarsten (1894) and subsequently investigated by Bang (1898-9, 1900-1), Feulgen (1919-20), E. Hammarsten (1920), Jones & Perkins (1924-5) and Steudel (1936), was shown by Jorpes (1924, 1928, 1934) to consist essentially of a ribopolynucleotide—probably a pentanucleotide and almost certainly similar to but not identical with the ribonucleic acid of yeast. It may form as much as 10 % of the weight of the pancreas, and, like yeast nucleic acid, contains uracil (Jorpes, 1924). The presence of a ribonucleic acid containing thymine in the mammary gland is suggested by Mandel & Levene (1905). As a result of this work, and of the work of Jones & Perkins (1924-5), who found that a ribonucleotide resembling the guanylic acid of the pancreas was also present in spleen and liver, and of similar observations by Thomas & Berariu

(1924), the suggestion, supported by Jorpes (1928), was made that ribonucleic acids might be widespread in animal tissue. They have more recently been demonstrated histochemically by Brachet (1933, 1937, 1940 *a, b*) in Amphibia, by Desclin (1940) in the anterior pituitary of the growing rat and guinea-pig, and by Painter & Taylor (1942) in toads' eggs. This work has also led to the view that nucleic acids are not entirely confined to the nucleus. As long ago as 1905 Beebe & Shaffer (1905) suggested that pentose derivatives might be present in the cytoplasm of tumour cells, and such work as that of Feulgen & Rossenbeck (1924), Feulgen, Behrens & Mahdihassan (1937) and of Behrens (1938) on rye embryos and yeast cells, of Delaporte (1939) on yeast, of Painter & Taylor (1942) on toads' eggs, and of Mirsky & Pollister (1942), has given rise to the conception that, while desoxyribonucleic acid is located exclusively in the nucleus (Mirsky, 1943), ribonucleic acid is chiefly a cytoplasmic constituent. It has been reported, however, in small amounts in the nucleus (Schultz, 1941) and especially in the nucleolus (Brachet, 1940 *a, b*; Caspersson & Schultz, 1940; Mitchell, 1942; Gersh, 1943).

Ribonucleic acids may occur in the form of phospholipin-ribonucleoprotein complexes in liver cells, e.g. in the mitochondria (Claude, 1941, 1943; Bensley, 1942), in normal chick embryonic tissue (Taylor, Sharp, Beard & Beard, 1942; Claude, 1940, 1941), in the embryonic chick brain (Taylor, Sharp & Woodhall, 1943), in tumour-producing fowl sarcoma extracts (Claude, 1939) and in the virus of equine encephalomyelitis (Taylor, Sharp, Beard & Beard, 1943). The importance of ribonucleic acids in biologically active material has further been emphasized by their presence in chloroplasts (Menke, 1938), in plant viruses (Loring, 1939; Stanley, 1939; Stanley & Knight, 1941), in the growth-promoting factor of embryo juice (Fischer, 1939, 1940, 1942), in some enzyme systems (Potter & Albaum, 1943) and by the presence of their derivatives in the materials liberated by injured cells (Loofbourow, 1942). Using spectrophotometric methods in conjunction with the quartz microscope, Caspersson (1936, 1940) has located materials which show powerful absorption at 2600 Å and which he claims to be ribonucleotides or ribonucleic acids (he does not distinguish clearly between mono- and polynucleotides), in the cytoplasm of rapidly proliferating cells such as sea-urchin eggs, the spinach root-tip periblem cell and the imaginal disks of larvae of *Drosophila melanogaster* (Caspersson & Schultz, 1939, 1940), in the embryo (Caspersson & Schultz, 1939; Caspersson & Thorell, 1941 *a, b*), in tumour tissues (Caspersson, Nyström & Santesson, 1941) and in the cells of actively secreting glands (Caspersson, Landström-Hydén & Aquilonius, 1941). A high concentration of similar absorbing materials, which may be ribonucleic acids, has been found in the cytoplasm of proliferating and differentiating cells exposed to X- or gamma-radiations (Mitchell, 1942). Caspersson (1941) has concluded that a high concentration of these absorbing materials (presumably ribonucleic acids or ribonucleotides) is characteristic of the cytoplasm of cells in which rapid protein synthesis is taking place (either for growth or for secretion), and is to be associated rather with protein synthesis than with metabolic activity or fermentation (Caspersson & Brandt, 1941).

We have attempted to establish first whether ribonucleic acids are indeed of widespread occurrence in animal tissues, and secondly whether there

is a high concentration of ribonucleic acid and/or of ribonucleotides in rapidly growing tissues. In a preliminary note (Davidson & Waymouth, 1943 *a*), we reported that ribonucleic acids are widely distributed in animal tissues. The comparison between embryonic and adult tissues is further extended in this paper.

METHODS

Materials

We have used chiefly the tissues from the freshly killed adult and embryo sheep. The embryos were all about 9–10 weeks old, a stage at which growth is very rapid (Gurlt, 1847). Arrangements were made to obtain the material immediately after slaughter but, even so, some hydrolysis by the very active tissue nucleotidases of nucleotide to nucleoside was unavoidable.

Small organs were pooled for analysis. In the case of large organs groups of specimens were analyzed separately at different times. Where fowl tissues were used, they were excised from the anaesthetized bird and dealt with immediately. Chick embryos were used immediately on removal from the egg. In some cases avian material was frozen with solid CO₂ immediately on removal from the bird or egg, but this procedure was later found to be unnecessary.

Analytical methods

Moisture was determined by drying at 100° to constant weight.

Nucleotide, nucleoside, and free-purine N were determined in trichloroacetic acid extracts by the method of Kerr & Blish (1932) as modified by Kerr (1940). The sum of all three gave the total acid-soluble purine N.

Total nucleic acid was estimated on 15–25 mg. portions on the basis of nucleoprotein phosphorus (NPP) determinations by the method of Berenblum, Chain & Heatley (1939), in which lipid P is extracted with ethanol-chloroform mixture, and acid-soluble P is extracted with HCl and the remaining acid-insoluble P, as determined by the micromethod of Berenblum & Chain (1938), is taken to be nucleic acid P. This is almost certainly not the case, as Jorpes (1928) has suggested, and our own observations (*vide infra*) support this view. Most methods of estimating NPP in tissues, such as the original one of Kossel (1882), and the later similar ones used by Malan & Green (1928), Needham & Needham (1930), Euler & Schmidt (1934), Tuttle, Erf & Lawrence (1941) and Marshak (1940), depend upon the same principle, and estimate residual P after extraction with lipid solvents and acid. In our results, therefore, the figures for NPP indicate rather residual acid-insoluble P (the 'protein-bound P' of Rosenthal & Drabkin (1943)). This fraction

will, of course, include phosphoprotein P (Plimmer & Scott, 1908; Plimmer & Kaya, 1910; Needham & Needham, 1930; Euler & Schmidt, 1934), which appears, however, to be low in nearly all tissues, including embryonic tissue (Euler & Schmidt, 1934). The residual P would also include such ill-defined compounds as the phosphoglucoproteins and gluconucleoproteins mentioned by Wells (1925).

Ribonucleic acid and desoxyribonucleic acid were estimated by methods involving the determination of pentose and of desoxypentose, after extraction and precipitation of the nucleic acids.

Acid-soluble P and lipid-soluble P were extracted from ethanol-ether-dried tissue and the resulting residue, containing the acid-insoluble P (protein-bound residual P as previously mentioned) was dried in a vacuum desiccator. Euler & Schmidt (1934) claim that preliminary treatment with ethanol and ether binds some acid-soluble P to the protein and renders its subsequent extraction difficult, but we have found no difference in the P content of powders prepared by Euler & Schmidt's and our own methods.

In extracting the nucleic acids from the tissue powders the use of alkali was avoided. Most methods for the isolation of desoxyribonucleic acid are sufficiently drastic to cause at least partial destruction of ribonucleic acid, but a suitable reagent for the extraction of both nucleic acids was found to be 10% NaCl (cf. Jorpes, 1928, 1934; Javillier & Allaire, 1926b, 1931). From the NaCl extract the nucleic acids were precipitated as lanthanum salts, which have been shown by Caspersson, Hammarsten & Hammarsten (1935) and by Caspersson (1936) to be very insoluble. From 10% NaCl solution the precipitation is incomplete unless one volume of ethanol is added. In the precipitate pentose and desoxypentose were colorimetrically estimated. We have sought independent confirmation of the presence of ribonucleic acids by more specific methods, using the enzyme ribonuclease which attacks specifically the ribonucleic acids (Kunitz, 1940). The action of ribonuclease is not fully understood. It does not break down nucleic acid completely to the component mononucleotides, but whereas 100% of the phosphorus of ribonucleic acid is precipitable by 0.25% uranyl acetate in 2.5% trichloroacetic acid, only 40% of the P is so precipitable after the action with the enzyme has gone to completion. The extent of the reaction depends on a number of factors (Kunitz, 1940), and for this reason the application of the enzyme to the exact quantitative estimation of the nucleic acids is not feasible. We have, however, made use of ribonuclease not only to confirm the presence of ribonucleic acids but to give a semi-quantitative estimation of the amount of nucleic acid present by allowing crystalline ribonuclease in

high concentration to act to completion on the NaCl extract and by measuring the amount of uranyl precipitable P rendered non-precipitable by the enzyme.

General analytical procedure. The fresh tissue is minced, dehydrated with several successive portions of ethanol and of ether, and dried in a vacuum desiccator. The dried material is ground to a fine powder in a mill and passed through a sieve. About 2 g. of the powder are weighed out into a stoppered centrifuge tube and are shaken for six 1 hr. periods with successive portions of about 40 ml. 0.1N-HCl. This process removes acid-soluble P, including simple nucleotides. The residue is washed twice with ethanol and is then extracted for two successive 2 hr. periods at 65° under a reflux condenser with an ethanol-chloroform mixture (3:1). It is then washed with ether and dried.

Total P is estimated in the extracted powder by a modification of the method of Allen (1940) and purine N by the method of Graff & Maculla (1935). Phosphoprotein P is determined by a method similar to that of Euler & Schmidt (1934), making use of the fact that phosphoprotein P is split off as inorganic P when the phosphoprotein is incubated at 37° for 24 hr. with 0.25N-NaOH (Plimmer & Scott, 1908). After removal of protein with trichloroacetic acid, inorganic P is precipitated, as described by Lohmann (1928), with Mathison's (1909) reagent.

For the nucleic acid extraction a suitable quantity (50–750 mg., depending on the P content) is then weighed out into a 15 ml. conical centrifuge tube and 2 drops triacetin added. Extraction with 10% NaCl is then carried out with five successive 3 ml. portions, (a) for 4 hr. at 0°, (b) overnight at 0°, (c) for 30 min. at 100°, (d) for 10 min. at 100°, and (e) for 10 min. at 100° respectively.

The extracts are combined and made up to 15 ml. 1.0 ml. is taken for total P estimation. 1.5 ml. portions are taken for the ribonuclease test (*vide infra*). 9 ml. are pipetted into a 25 ml. centrifuge tube and 1 ml. 2% lanthanum acetate and 10 ml. ethanol added. After 1 hr. at 0° the precipitate is centrifuged down and washed twice with 3 ml. 0.2% lanthanum acetate. It is decomposed with 0.6 ml. 0.5M- Na_2CO_3 and 5.4 ml. water are added. The lanthanum carbonate is centrifuged down.

The supernatant is treated as follows: (i) 1 ml. is taken for total P estimation; (ii) 1 ml. is taken for estimation of pentose by a modification of the method of Reeves & Munro (1940). This method depends upon the liberation of furfural, which is trapped in xylene and allowed to react with aniline acetate. The 1 ml. of solution is pipetted into a graduated test-tube with a B 14 ground glass socket. 2 ml. 5.55N-HCl and 5 ml. xylene (purified by distillation) are added. The tube is attached to an

air condenser with ground-glass cone and is immersed in a briskly boiling water-bath for $2\frac{1}{2}$ hr. The contents are cooled and the xylene layer made up to 7 ml. The lower aqueous layer is sucked off with a fine pipette and the xylene layer dried with anhydrous sodium acetate. When the xylene has cleared, 5 ml. is pipetted into a dry pyrex tube and 5 ml. freshly made aniline acetate reagent (1 ml. colourless aniline dissolved in 50 ml. Analar glacial acetic acid and 50 ml. ethanol) added. The tube is allowed to stand in the dark room for precisely 20 min. and the red colour then examined in the Hilger Spekker Absorptiometer (filter no. 6), with a calibration curve drawn up in terms of ribonucleic acid phosphorus (RNAP) and prepared from standard solutions of ribonucleic acid purified by precipitation from glacial acetic acid as described by Kunitz (1940). Yeast ribonucleic acid and liver ribonucleic acid gave similar curves. Desoxyribonucleic acid does not interfere. (iii) 3 ml. are taken for the colorimetric estimation of desoxypentose by the modification by Sevag, Smolens & Lackman (1940) of the diphenylamine reaction of Dische (1930). The 3 ml. solution are pipetted into a conical graduated centrifuge tube containing 1 ml. 0.55 N-HCl. The tube is heated in a boiling water-bath for 15 min., cooled, and the volume made up to 4 ml. 3 ml. of the supernatant are transferred to a pyrex tube and 8 ml. freshly made diphenylamine reagent added (1 g. diphenylamine (Analar, twice recrystallized from ethanol) dissolved in 2 ml. H_2SO_4 (Analar) and 98 ml. glacial acetic acid (Analar)). The tube is placed in the briskly boiling water-bath for 3 min., quickly cooled and, after 5 min., the blue colour is read in the Hilger Spekker Absorptiometer (filter no. 1) and compared with data in a calibration curve drawn up in terms of desoxyribonucleic acid phosphorus (DNAP). Ribonucleic acid does not interfere, nor does chondroitin sulphuric acid. Using these two methods, we have obtained good recovery from a mixture of ribo- and thymonucleic acids in 10% NaCl. Both calibration curves were made with nucleic acids precipitated as La salts.

For the ribonuclease method, 1.5 ml. NaCl extract are pipetted into each of three 15 ml. conical centrifuge tubes. 2.4 ml. veronal buffer pH 6.12 (Michaelis, 1931) are added to each tube and 0.2 ml. of a solution of 5 mg. crystalline ribonuclease in 100 ml. water is added to one tube. The other two tubes are used as controls. To one of these are added immediately 4.0 ml. 0.25% uranyl acetate in 2.5% trichloroacetic acid. The remaining control tube and the tube containing enzyme are immersed in a thermostatic bath at 37° for 1 hr. before the uranyl acetate reagent is added. 15 min. after addition of the reagent the tubes are centrifuged and the precipitate washed with 2 ml. 0.125% uranyl acetate

in 1.25% trichloroacetic acid. It is then dissolved in 0.5 M- Na_2CO_3 and the solution is transferred to a digestion flask for total P determination. The non-incubated tube gives the amount of uranyl precipitable P originally present, while the tube containing enzyme gives the amount present after ribonucleic acids have been broken down. The second control without enzyme gives a measure of the amount of spontaneous decomposition of nucleic acid in 60 min. at 37° . In most cases it is low.

With each batch of tubes used in this determination a standard set of three tubes containing 0.1% purified ribonucleic acid in 10% NaCl is set up to check the extent of hydrolysis. Under the conditions stated, about 60% of the ribonucleic acid P is rendered non-precipitable by the enzyme. Sodium chloride does not inhibit the action of the enzyme, but the degree of hydrolysis of ribonucleic acid may be to some extent influenced by the quantity of desoxyribonucleic acid present. The enzyme has no action on desoxyribonucleic acid.

Examination of the procedure. When calibration curves reading directly in ribo- or desoxyribonucleic acid phosphorus are used, the assumption is made that the nucleic acid in the tissues contains the same amounts of ribose (or rather furfural-yielding material), since purine and pyrimidine nucleotides yield different amounts of furfural (Levene & Jorpes, 1930) relative to P, or of desoxyribose to P, as do the pure acids used to prepare the calibration curves. This assumption appears to be justified in the case of a ribonucleic acid which we have isolated from the liver and which gives figures which agree well with the calibration curve prepared from yeast nucleic acid. In the case of desoxyribonucleic acid (Vowles, 1940), the sugar residues from the purine nucleotides also react in the estimation to a much larger extent than those from pyrimidine nucleotides, and the estimation of desoxyribose will be valid only if all tissue desoxyribonucleic acids contain equimolecular amounts of purine and pyrimidine. This appears to be the case (Mirsky, 1943).

Compounds of lanthanum and other metals are capable, under certain conditions, of hydrolyzing organic phosphates (Bamann & Meisenheimer, 1938). The danger of interference from actions of this type in the present circumstances is negligible.

One object of extracting the nucleic acids and precipitating them as lanthanum salts was to reduce the possible danger of interference in the pentose estimation by furfural derived from uronic acids (e.g. of muco- and sulpho-polysaccharides).

It cannot be taken for granted that the ribonucleic acids of the tissues are identical with yeast nucleic acid. They do, however, function as substrates for ribonuclease, and the ribonucleic acid which we have isolated from liver suffers the same degree of hydrolysis as does yeast nucleic acid.

RESULTS

Total nucleic acid

The results of the NPP estimations are shown in Table 1.

The position of nervous tissue is rather striking (Table 1). Not only does the white matter of the central nervous system have a higher NPP than the grey matter, but even peripheral nerve has an appreciable NPP content comparable indeed with that of muscle. Caspersson (1941) records a high

Table 1. *Nucleoprotein phosphorus (NPP) and water contents of adult and embryonic tissues*

Sheep embryos, 9-10 weeks. Chick embryos, 16 days.

Each figure represents the mean value of several estimations from different animals.

NPP on dry-weight basis calculated from NPP on wet-weight basis and water content.

Tissue	Adult			Embryo			Ratio	
	Water content (%)	NPP content (mg./100 g.)		Water content (%)	NPP content (mg./100 g.)		Ratio	Embryo NPP / Adult NPP
		Fresh wt.	Dry wt.		Fresh wt.	Dry wt.		
Sheep								
Liver	69.7	70	231	82.6	103	592	1.5	2.6
Lung	79.0	88	419	89.0	84	763	1.0	1.8
Small intestine	80.3	110	559	88.4	78	673	0.7	1.2
Heart	77.7	29	129	87.0	64	492	2.2	3.8
Spleen	77.2	129	566	83.0	109	642	0.8	1.1
Kidney (cortex)	81.0	80	421	87.0	85	654	1.2	1.6
Kidney (medulla)	85.5	47	324	90.4	34	354	0.7	1.1
Brain (grey matter)	84.2	18	114	92.6	24	324	1.3	2.8
Brain (white matter)	74.2	53	206	90.5	24	253	0.5	1.2
Muscle	74.8	19	75	87.4	47	373	2.5	5.0
Cartilage	60.1	21	53	78.4	43	199	2.0	3.8
Testis	86.4	58	427	—	—	—	—	—
Ovary	81.9	101	558	—	—	—	—	—
Thyroid	75.4	25	102	80.2	78	394	3.1	3.9
Thymus*	—	—	—	84.2	170	1080	—	—
Skin	63.5	38	107	89.3	56	524	1.4	4.9
Placenta	87.8	59	484	—	—	—	—	—
Lymph node (calf)	79.6	185	925	—	—	—	—	—
Fowl								
Heart	80.1	32	161	91.0	75	835	2.3	5.2
Liver	72.3	113	408	80.0	95	475	0.8	1.2
Brain	79.1	25	119	89.1	50	459	2.0	3.9
Nerve	71.2	59	205	—	—	—	—	—
Muscle	75.9	30	125	81.4	74	398	2.5	3.1
Rous sarcoma	88.2	61	516	—	—	—	—	—

* 15-week embryo.

The water content of the embryonic tissues is considerably higher than that of the corresponding adult tissues. As a result the difference in NPP between embryo and adult is much more marked when the comparison is made on a dry-weight basis than on a fresh-tissue basis.

When the figures for the embryo and the adult are compared on a dry-weight basis it is found that the NPP in the embryo is invariably higher than in the corresponding adult tissue. In some cases the difference is very marked, e.g. heart, muscle, cartilage and thyroid. In some tissues, on a fresh-weight basis, the difference between embryo and adult is small and the figure for the adult may actually be higher, e.g. intestine, spleen, kidney medulla, brain (white matter).

ribonucleotide or ribonucleic acid content in nerve cells.

The high water and NPP contents of Rous sarcoma (Table 1) appear to be characteristic of neoplastic tissue.

The figures for the P content of the tissues extracted in bulk (Table 2) are of course essentially the same as the NPP figures on a dry-weight basis, except that the P content is expressed in terms of a powder from which all acid-soluble and lipid material has been removed. To investigate whether or not all this P could be taken to be nucleic acid P, purine estimations were carried out on the extracted powders. If the tissue nucleic acids have the same relative amounts of purine and P as have purified thymonucleic acid and yeast nucleic acid (and this

Table 2. *Analyses of tissue powders remaining after extraction of acid-soluble and lipid P, and of lanthanum salts precipitated from the NaCl extract of these powders*

All tissues from the adult or 9-10 weeks' embryo sheep unless otherwise stated.

Nucleic acid P = purine N $\times 0.886$ (assuming purine N : P atomic ratio of 10 : 4).

DNAP = desoxyribonucleic acid P. RNAP = ribonucleic acid P.

Tissue	Extracted tissue powder				Precipitate of La salts				
	Total P (mg./ 100 g.)	Purine N (mg./ 100 g.)	Nucleic acid P calcu- lated from purine content (mg./ 100 g.)	Nucleic acid P as per- centage of total P	P in NaCl extract as per- centage of P in ex- tracted powder	P in La ppt. as per- centage of P in NaCl extract	DNAP as per- centage of P in La ppt.	RNAP as per- centage of P in La ppt.	Ratio RNAP DNAP
Adult liver	384	353	313	82	84	74	19	65	3.5
Embryo liver	1175	1241	1101	94	75	60	24	61	2.6
Adult lung	565	397	352	62	94	73	50	43	0.9
Embryo lung	1225	866	767	63	93	69	50	35	0.7
Adult gut	636	622	551	87	88	66	41	39	1.0
Embryo gut	958	782	693	72	82	83	31	35	1.1
Adult heart	219	174	154	70	56	65	19	70	3.6
Embryo heart	616	546	484	79	81	79	25	59	2.4
Adult spleen	914	974	863	95	78	67	50	27	0.5
Embryo spleen	1380	1448	1284	93	97	70	47	26	0.6
Adult kidney	442	400	355	80	74	74	29	52	1.8
Embryo kidney	923	949	840	91	79	72	49	41	0.8
Adult brain	369	215	190	52	40	67	31	65	2.1
Embryo brain	556	340	301	54	82	79	23	55	2.3
Adult muscle	147	145	129	88	55	71	19*	66	3.6*
Embryo muscle	650	572	507	78	95	70	37	53	1.4
Adult cartilage	84	85	75	89	63	40	41	—	—
Embryo cartilage	382	343	304	80	92	53	40	60	1.5
Adult thyroid	166	167	148	89	63	68	24	48	2.0
Embryo thyroid	682	732	649	95	83	69	39	41	1.1
Adult skin	135	141	125	93	68	48	32	60	1.9
Embryo skin	842	824	730	87	94	68	43	41	1.0
Adult testis	660	624	553	84	70	66	26	67	2.6
Adult ovary	484	444	394	81	87	42	39	52	1.3
Sheep placenta	655	613	543	83	83	74	33	65	2.0
Thymus (15-week sheep embryo)	2760	2696	2390	87	97	72	66	13	0.2
Whole blood (embryo sheep)	152	—	—	—	63	73	32	63	2.0
Nuclei (embryo sheep liver)	2630	2382	2110	80	86	82	75	14	0.2
Dried sheep embryo extract	192	175	155	81	82	93	11	83	7.4
Nucleoprotein fraction from embryo extract	658	746	661	101	82	80	81	10	8.0
Whole chick embryo (9 days)	1040	1017	901	87	86	74	29	58	2.0
Whole human embryo (3 months)	590	613	544	92	80	75	29	59	2.1
Ox pancreas	1670	1782	1580	95	79	87	10	82	8.0

* The total nucleic acid content of muscle is so low that the figures quoted should be regarded as provisional.

qualification appears to hold for the desoxyribo-nucleic acids of the tissues (Mirsky, 1943), the atomic ratio of purine N to P is 10 : 4 for tetra-nucleotides. The ratio would, of course, be higher in the case of pentanucleotides (Jorpes, 1934). As Table 2 shows, in many cases the figure for nucleic acid P calculated from purine N is rather less than the figure for total P. It is probable therefore that

some of the P in the extracted powder is not present in the form of nucleic acid. Alders (1927) carried out estimations of both NPP and total purine in thymus, kidney, pancreas and spleen. Except in the case of ox pancreas, the nucleic acid calculated from the purine content was lower than that calculated from the NPP (see also Graff & Barth, 1938).

The nature of the non-nucleic acid residual P is still in doubt. Some of it may be present as phosphoprotein, but estimations show that the phosphoprotein P is in most cases negligible and in all cases is less than 5% of the total residual P except in adult brain (6.2%) and adult heart (6.7%). Euler & Schmidt (1934) found that the phosphoprotein P in most tissues is very low, and in so far as comparison is possible (Euler & Schmidt quote only for fresh tissue) our figures are rather lower than theirs.

Ribonucleic and desoxyribonucleic acids

The percentage of P extracted by NaCl from different tissues is not uniform (Table 2). In most cases it is of the order of 80–90%, but in certain tissues, e.g. adult heart, adult muscle and adult brain, particularly the last, the amount extracted is low, although constant for different specimens.

The amount of P in the NaCl extract, which appears in the precipitate of La salts, is also variable (Table 2). Precipitation is poor in the case of cartilage, skin and ovary, all of which yield extracts containing much material of a mucopolysaccharide type. As a result the La precipitate contains in most cases 60–80% of the P of the extracted powder. As previously shown, the P of the extracted powder is not entirely derived from nucleic acid. In such tissues as adult heart, brain, muscle, cartilage, skin and ovary, the percentage is much lower. It is, however, even in these cases remarkably constant for different batches of powder prepared from different animals.

Results of the analyses of the La precipitate for pentose and desoxypentose are shown in Table 2 expressed in terms of ribonucleic acid phosphorus (RNAP) and desoxyribose nucleic acid phosphorus (DNAP) as percentages of the total P. In most cases 80–95% of the total P in the La precipitate can be accounted for in terms of ribo- or desoxyribonucleic acid. In the case of adult cartilage, the sum was greater than 100%, on account of interference in the pentose estimation by chondroitin sulphuric acid. The RNAP/DNAP ratios for the La precipitates (Table 2) vary greatly from tissue to tissue, being high in such tissues as pancreas, liver, heart, brain, testis and muscle and low in thymus, spleen and lung. These ratios are not necessarily the same as those which obtain in the original tissue, especially in those cases where the proportion of P extracted and precipitated is relatively low, e.g. adult heart and adult brain.

In any given tissue the ratio of RNAP to DNAP is of the same order in the embryo as in the adult, or is somewhat higher in the adult. Therefore, since the amount of total nucleic acid is higher in the embryo than in the adult, any given embryonic

tissue must have a higher content not only of nuclear desoxyribonucleic acid, but also of cytoplasmic ribonucleic acid.

To examine the view that desoxyribonucleic acid is found in the nuclei and ribonucleic acid in the cytoplasm, nuclei of embryo sheep liver were isolated by the citric acid method of Marshak (1941). Acid-soluble and lipoid P were extracted from the nuclei and the residue worked up in the usual way (Table 2). The very low RNAP/DNAP ratio (0.2) suggests that, if the nucleic acid content of the nuclei remains unaltered during the isolation process, there is indeed very little ribonucleic acid in the nuclei. The small amount found may be due in part at least to adherent cytoplasmic residues. On the other hand, ribonucleic acid predominated in a saline extract of minced whole sheep embryo. The extract was prepared by allowing the minced embryos to stand overnight with Tyrode solution at 0°. It was then filtered through gauze and centrifuged. The extract so obtained had powerful growth-promoting action on chick heart fibroblasts *in vitro* (Davidson & Waymouth, 1943*b*). It was dried either in vacuum desiccators over H₂SO₄ or in the air at room temperature in the blast of an electric fan as recommended by Fischer (1942), and powdered. Such a saline extract would be expected to contain mainly cytoplasmic material (cf. Mirsky & Pollister, 1942), and the RNAP/DNAP ratio obtained (7.4) is evidence in favour of the predominance of ribonucleic acids in the cytoplasm.

From such an embryo extract the 'nucleoprotein' fraction which was precipitated by acetic acid at pH 4.3 was centrifuged down, washed repeatedly with very dilute acetic acid, extracted with ethanol-chloroform mixture and worked up as usual. Again the ratio was high (8.0).

Confirmatory evidence for the presence of ribonucleic acids by experiments with ribonuclease

In all cases the NaCl extract of the tissue contained a proportion of phosphorus which treatment with ribonuclease rendered non-precipitable by uranyl acetate reagent. The amount of P in the NaCl extract precipitable by the uranyl acetate reagent is in most cases of the same order as that precipitable by lanthanum acetate. With yeast ribonucleic acid as substrate, 60% of the P is rendered non-precipitable when ribonuclease has acted to completion. On the same basis the ribonucleic acid content of the NaCl tissue extract has been calculated as a percentage of the total P in the extract. The relationship between the percentage of RNAP in the La precipitate and in the NaCl extract is shown in Fig. 1. In most cases there is reasonably good correlation between the pentose content in the La precipitate and the amount of substrate for

ribonuclease in the NaCl extract. This agreement gives independent confirmation of the presence of ribonucleic acids in the tissue powders.

Fig. 1. Correlation between the ribonucleic acid P content of the tissues, calculated from the pentose content and from the ribonuclease test.

Nucleotides, nucleosides, and free purine

The simple mononucleotides differ from the polynucleotides in being acid-soluble and in appearing in a trichloroacetic acid filtrate. The purine nucleotides which are precipitated by uranyl acetate include adenosine triphosphate, adenylic acid, inosinic acid, the nicotinamide nucleotides and riboflavin-adenine-dinucleotide. Of these adenosine triphosphate accounts for the major part of the total tissue nucleotides, while the last two form only a very small proportion of the total. The nucleosides together with free purines are estimated separately, and the sum of these and the nucleotides gives the total acid-soluble purine N. As most tissues (particularly brain) contain an active nucleotidase which rapidly dephosphorylates nucleotides to nucleosides (Reis, 1937; Kerr, 1942), and as rapid deamination of adenine derivatives is liable to occur, the tissues must be ground in trichloroacetic acid immediately on removal from the animal. For the sheep tissues, in which some hydrolysis of nucleotide to nucleoside was unavoidable, figures for total acid-soluble purine N only are quoted (Table 3). Control experiments have shown that the loss in total acid-soluble purine under the conditions of our experiments would be very slight. By the method of analysis employed excellent agreement of duplicates was obtained, but specimens of the same tissue from different animals showed a wide range of variation. Individual figures are therefore quoted. Of the tissues examined, adult muscle gives higher figures than any other

tissue. Lower values were found for all the embryo tissues than for the corresponding adult tissues (Table 3).

Table 3. *Total acid-soluble purine N (nucleotide N + nucleoside N + free purine N) in trichloroacetic acid extracts of different samples of adult sheep and 9-10 weeks' embryo sheep tissues*

Tissue	Total acid-soluble purine N (mg./100 g. fresh tissue)	
	Embryo	Adult
Liver	24.4, 28.6, 28.0	35.9, 36.4
Heart	30.9, 20.0, 21.7	37.5, 48.3, 45.9, 43.8
Muscle	24.2, 25.4, 25.3	53.9, 40.6, 57.1
Spleen	30.8	41.5, 29.8, 37.8, 35.0
Brain:		
Whole	20.9, 21.6, 18.6	24.6, 32.6
Grey matter		20.0, 28.0, 17.5
White matter		23.8, 23.1, 19.6
Kidney	22.3, 17.5, 17.5	25.3, 31.5
Lung	24.2, 13.3, 15.4	30.5, 25.7
Cartilage	15.6, 19.6, 15.4	—

In the case of the fowl tissues (Table 4), the conditions of the experiments minimized the chances of hydrolysis of nucleotide to nucleoside, and figures for nucleotide and nucleoside are quoted separately. Adult muscle has the highest total nucleotide content. In muscle, however, the amount of nucleoside relative to nucleotide is low; in heart, and especially in liver, nucleoside and free purine form a much more significant proportion of the total acid-soluble purine. In the chick embryo both fractions are low and the total acid soluble purine N content is of the same low order as in the case of the sheep embryo.

Our results are expressed in terms of fresh tissue. Owing to the higher water content of embryonic tissue, much smaller differences between embryo and adult are apparent if the results are expressed in terms of dry weight.

Preliminary results on tumour tissues suggest that the total soluble purine N is low. Figures for the Rous sarcoma are shown in Table 4.

DISCUSSION

Estimations of the nucleic acid content of sheep tissues have not, as far as we are aware, been previously recorded. Kossel (1882) quotes only one figure for sheep tissue (brain) and his figure (99 mg./100 g. of fresh tissue) in this case, as in the case of other tissues from other species, is rather higher than ours. Our figures are, however, very much of the same order as those of Javillier & Allaire (1926*a*) for the horse, Jorpes (1928) for the ox and Grund (1910) for the dog and hen.

On a dry-weight basis embryonic tissues are richer in nucleic acid than the corresponding adult

Table 4. *Nucleotide, nucleoside, and free purine content of the adult and embryo fowl*

Adult birds fasted 24 hr. before use. All figures are in mg. N/100 g. fresh tissue.

Exp. no.	Tissue	Nucleotide N	Nucleoside N (free purine N)	Total soluble purine N
204*	Adult liver	21.6	14.1	35.7
208	"	30.0	16.5	46.5
209	"	20.3	13.6	33.9
212	"	26.6	23.8	50.4
219	"	27.3	14.3	41.6
203*	Adult heart	27.6	12.0	39.6
207	"	29.1	15.4	44.5
210	"	23.4	15.0	38.4
201*	Adult muscle (breast)	52.4	5.9	58.3
205	"	42.3	10.2	52.5
402†	"	40.0	11.2	51.2
404†	"	31.1	13.7	44.8
406†	"	41.8	11.6	53.4
202	Adult muscle (leg)	34.6	7.3	41.9
206	"	26.3	7.7	34.0
408†	"	28.7	9.1	37.8
301*	Whole chick embryo (9 day)	14.1	6.0	20.1
308	" (9 day)	10.8	6.0	16.8
1 E	" (10 day)	11.2	5.6	16.8
2 E	" (10 day)	12.6	7.0	19.6
302*	" (13 day)	14.8	3.6	18.4
303*	" (13 day)	14.4	4.1	18.5
305	Chick embryo muscle (16 day)	15.8	7.0	22.8
9 E	Chick embryo viscera (16 day)	16.8	9.8	26.6
8 E	Chick embryo minus viscera (16 day)	17.5	7.0	24.5
401†	Rous sarcoma	11.9	7.0	18.9
403†	"	7.7	7.0	14.7

* Tissues frozen in solid CO₂.

† From birds with Rous sarcoma.

tissues. No extensive comparison of the tissues of any one species is to be found in the literature, but figures have been quoted for individual tissues. Masing (1911) has shown that the NPP of whole-rabbit embryo and of embryo rabbit liver diminishes progressively as the age of the embryo increases, while Dumm (1943) has recorded a fall in the NPP of embryo rat liver with age. Le Breton & Shaeffer (1923), using pig and mouse embryos, have found a fall in purine N concentration as gestation advances. Kossel (1882) quotes a higher figure for cattle embryo muscle than for adult muscle.

Liver tissue is of special interest. Kosterlitz & Cramb (1943) have shown that in rats fasted 24–48 hr. there is a fall in liver NPP in terms of the original body weight, although the NPP/100 g. of liver rises. We have confirmed this rise in NPP in the liver of fasting rat. It would appear, therefore, that in the liver at least, the NPP is conditioned to some extent by the nutritional condition of the animal. Embryo liver differs from adult in containing numerous erythropoietic cells, and chemical comparison of embryo and adult liver must be made with this reservation in mind.

The procedure which we have described must not be regarded as an accurate quantitative estimation of the ribonucleic acid content of tissues. It serves,

however, to demonstrate the presence of ribonucleic acids in all the tissues examined and to give an indication of the relative amounts of ribo- and desoxyribonucleic acids.

If crystalline ribonuclease attacks ribonucleic acids only, and if it is uncontaminated by traces of other enzymes (and there appears to be no reason to doubt the validity of these assumptions), then the NaCl extract of the tissue powder contains a substrate for ribonuclease, presumably therefore a ribonucleic acid. Moreover, in the precipitate of lanthanum salts obtained from the NaCl extract, a certain proportion of the P present can be accounted for in terms of pentose. Finally, the amount of pentose-containing material and the amount of substrate for ribonuclease show a definite correlation. There seems to be no doubt, therefore, that we are dealing with some type of ribonucleic acid. Whether all tissues contain the same ribonucleic acid and whether in that case it is similar to the ribonucleic acid of the pancreas or of yeast or to neither, is of course unknown, but preliminary observations on the ribonucleic acid which we have isolated from liver suggest that it is similar to yeast nucleic acid.

The amounts of ribonucleic acid in many tissues are high. Where the same organs have been ex-

amined by Jorpes (1928) there is agreement between his results and our own. Jorpes estimated both NPP and total pentose in the fresh tissue. For thymus he found a total NPP of 0.441 % and a pentose content of 0.152 % corresponding to 0.063 % pentose nucleic acid phosphorus (if all pentose comes from ribonucleic acid). 14.2 % of the NPP could therefore be accounted for as RNAP. Similarly, in pancreas 84.3 % of the NPP is RNAP. If the remainder of the NPP is all DNAP these figures would give ratios RNAP/DNAP of 0.2 for thymus and 5.4 for pancreas. From Jorpes's data the ratios can similarly also be calculated to be 2.1 for ox liver, 3.2 for rabbit liver, and 0.5 for ox spleen. The ratios calculated from the results of Jorpes, therefore, are of the same order as our own.

In the case of liver nucleoprotein, Brues, Tracy & Cohn (1942), using the radioactive phosphorus isotope P^{32} , found that only about 50 % of the residual P after extraction of acid-soluble and lipid P could be accounted for in terms of desoxyribonucleic acid. The remainder had a higher specific activity. These authors point out that they do not distinguish cytoplasmic from nuclear nucleic acids but that they have obtained some evidence that 'the former have a higher rate of phosphorus turnover than the latter'. Somewhat similar results were obtained with P^{32} by Hevesy & Ottesen (1943), who have shown that after very exhaustive extraction of muscle and other tissue of the frog with trichloroacetic acid and ethanol-ether, the specific activity of the residual P was much higher than that of the P in purified (desoxyribo-) nucleic acid prepared from the same material. These results might be due in part to the presence of ribonucleic acids with a high specific activity.

Fischer (1939, 1940, 1942) has claimed that the growth-promoting substances of embryo juice are associated with the nucleoprotein fraction and has suggested that the activity may lie in ribonucleoproteins. Our results show that of this fraction some 80 % is in fact ribonucleoprotein, but its relationship to the growth-promoting properties of the extract is not fully established.

The acid-soluble nucleotide content varies considerably from tissue to tissue and even, in contrast to total nucleic acid, in different specimens of the same tissue. For comparison there are few references in the literature to the nucleotide content of tissues and none to sheep tissues. Our results, however, are of the same order as those quoted by Kerr (1940, 1942) for the brain, muscle and liver of the dog, and by Barrenscheen & Peham (1941) for a number of tissues from a variety of different sources including fowl muscle. The figures of Dell'Aqua (1935) for rabbit liver are appreciably higher than our values for sheep or fowl liver.

From our results it is apparent that in embryonic tissues which are growing rapidly there is no high concentration of acid-soluble purine nucleotides. On the other hand, the total nucleic acid of an embryonic tissue is higher than that of the corresponding adult tissue and since the ratio of ribonucleic acid to desoxyribonucleic acid is of the same order in the embryo as in the adult, a high concentration of cytoplasmic ribopolynucleotides is to be expected in the embryonic tissue. This might explain the high power to absorb light of wavelength 2600 Å found by Caspersson and his colleagues in the cytoplasm of rapidly growing tissues.

Data so far obtained from neoplasms suggest that tumour tissue resembles embryo tissue in possessing a low soluble nucleotide content and a high polynucleotide content. Barrenscheen & Peham (1941) quote figures for a number of miscellaneous tumours in which the total soluble purine is almost invariably low, while the total purine (including nucleic acids) is high.

These results tend to show that rapidly growing tissues have in general a low concentration of total soluble purine (including nucleotides) and that the high concentration of cytoplasmic 'nucleotides' reported by Caspersson is not due to the presence of acid-soluble nucleotides.

It is of interest to note that Stedman & Stedman (1943b) have recently reported that the nuclei of rapidly growing tissues, such as chick embryo and mouse and rat carcinoma, are characterized by a much lower histone content than is found in non-proliferating tissues. It is probable that the high content of both desoxyribo- and ribonucleic acid in rapidly growing tissues is intimately connected with the process of cell division. As the result of experiments on tissue cultures growing in embryo extract, Willmer (1942) has suggested that a rise in total nucleic acid may precede cellular division by some hours, and our own (unpublished) observations support this view.

The relationship between nucleotides and nucleic acids in tissues is uncertain. Although the function of many of the nucleotides is well known, that of the nucleic acids is obscure. Ostern, Terszakowec & Hubl (1938) have brought forward evidence that, in yeast, ribonucleic acid may act as a reservoir from which nucleotides including adenosine triphosphate are obtained, while Brachet (1937) has claimed that, in the development of the sea urchin egg, ribonucleic acid is converted into desoxyribonucleic acid. The synthesis of desoxyribonucleic acid from ribonucleotides by rapidly proliferating cells is, according to Mitchell (1942), inhibited by X- and γ -radiation, with consequent accumulation of cytoplasmic ribonucleotides or more probably of ribonucleic acids. Whether or not nucleotides are

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The 'Activation' of Phosphatase

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Following the demonstration that ligation of the common bile-duct led to an enormous increase in the amount of phosphatase in the blood (Armstrong, King & Harris, 1934), we investigated the possibility that the enhanced plasma phosphatase values found in jaundice and certain bone diseases might be due to the presence of a powerful activator rather than to an actual accumulation of phosphatase in the blood stream. Since none of our

experiments gave evidence of the first possibility the results were not published, but in view of a renewal of interest in the subject and of several claims to have demonstrated an activator (Thannhauser, Reichel, Gratton & Maddock, 1938; Cantarow, 1940), it seemed advisable to complete our observations and report the conclusions.

Our first experiments, following the observation by Armstrong *et al.* (1934) of large amounts of