Phosphorus Compounds in the Cell Nucleus

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Section 1. Introduction.

1.1. Historical.

The theory that all animal and plant organisms are composed of discrete units known as cells was developed during the first half of the nineteenth century. That a small body, the nucleus, was almost universally contained within the cell became known in the latter half of this period, initially through the work of Brown. A little later (1848) Hofmeister gave a detailed description of the chromosomes from the pollen mother cells of Tradescantia. However, the belief that the chromosome content of all the nuclei in an organism is identical did not arise until the present century.

Almost forty years after the discovery of the cell nucleus Miescher began his investigations into its chemical nature in the laboratory of Hoppe-Seyler. In 1869 he announced the separation from the nuclei of pus cells of a new and abundant protein containing a high percentage of phosphorus. Papers subsequently appeared from Hoppe-Seyler's laboratory describing similar materials from yeast, the red blood cells of birds and reptiles, and a fraction of casein.

Miescher (1897), continuing his work in Basel on the nuclear material of salmon sperm, discovered that "nuclein" (nucleic acid) existed in the cell as a salt-like

compound with a base which he named protamine. He recognised that "nuclein" was a polybasic compound and its non-protein nature.

Attention was early directed to the components of nucleic acid and preliminary work by Miescher and by Kossel led to the recognition of adenine, guanine and a carbohydrate moiety as constituents. The early part of the present century saw the development of the chemistry of the nucleic acids. The important task of preparing the new compounds, first tackled by Altmann, was taken up by Kossel and Newmann, Schmiedeberg, Levene, and Feulgen, and the investigation of hydrolysis products proceded. Through the work of Miescher, Kossel, Levene, Osborne and Harris, Jones, and Steudel it was shown that adenine, guanine, thymine and cytosine were constituents of a type of nucleic acid first isolated from the thymus gland of animals and named thymonucleic acid. The unusual sugar present was eventually shown to be deoxyribose by Levene and this type of nucleic acid is now generally called deoxyribonucleic acid (DNA). The investigations of Steudel, and of Levene and Mandel, in particular, led to the conception that the four bases were present in equimolar proportions.

A second type of nucleic acid first isolated from yeast and thought to be characteristic of plants was shown to contain the bases adenine, guanine, cytosine and uracil

which were apparently equally represented in the molecule. The yeast nucleic acid differed from the thymus type in containing a pentose sugar now known to be d-ribose and a nucleic acid of this type is usually referred to as ribonucleic acid (RNA).

The early work on the chemistry of the nucleic acids has been reviewed in detail by Jones (1920) and Levene and Bass (1931).

Following the discovery of protamine in salmon sperm by Mieschcr, Kossel (1928) obtained a protein, rich in nitrogen, from bird erythrocytes which was in salt-like combination with nucleic acid. He called it histone and considered it analogous to the protamine of Mieschcr. A similar material was obtained from calf thymus by Lilienfeld and the complex of this histone with nucleic acid was studied by Huiskamp.

The relationship between the histones and protamine was strongly emphasized by work on the base obtained from sturgeon sperm. Kossel found it to be similar to but not identical with salmon protamine. Like histone it contained all three basic amino acids. Bases obtained from the sperm of other fish showed similarities to salmon protamine and to histone (see Table 1). Recent work by Stedman and Stedman (1944) on the salmon emphasizes the relationship. They have verified the presence of protamine in the sperm head and

Table 1.

The Amino Acid Composition of the Protamines and Histones

(from Kossel, 1928),

(figures refer to amino acid N as % total N).

	Protamines			Transition form	Histones	
	Salmine	Percine	Sturine	Sardinia coerula	Cod	Calf Thymus
Arginine	89.2	78.1	67.4	27.8	26.9	27.1
Histidine		5.6	10.1	23.0	3.3	5.8
Lysine			7.5	5.5	8.5	9.7
Glycine						+
Alanine			+			+
Leucine Isoleucine			+			+
Valine	1.65					
Serine	3.25					
Cystine				0.6		
Glutamic acid						+
Phenyl-alanine						+
Tyrosine					+	+
Tryptophan					+	
Proline	4.3					+
Monoamino acids		9.8				

shown that in the nuclei of salmon liver and erythrocytes it is replaced by histone.

Largely through the work of Kossel (1928) evidence accumulated to confirm the protein-like nature of the protamines. He subdivided them thus: (type I) those containing arginine as the only basic amino acid; (type II) those containing arginine and either histidine or lysine; (type III) those containing all three basic amino acids. The presence of other amino acids (i.e., valine, proline, serine, alanine, tyrosine and tryptophan) was subsequently demonstrated. Kossel also suggested "protamine" as a general name for the sperm bases, the family name of the fish from which they were obtained being used in individual cases (e.g., sturine).

The protamines are characterised by their relatively low molecular weight, simple amino acid composition and marked basic properties which are due to the extremely high proportion of basic amino acids present (see Table 1).

The original work of Miescher on the salmon seemed to indicate that a second protein was present in the sperm heads although Schmiedeberg's interpretation (see Miescher, 1897) of his analytical data suggested that the sperm head consisted almost entirely of protamine and nucleic acid. (For a discussion of this point see Stedman, 1944).

The work of Mathews and of Steudel on herring sperm

seemed to confirm Schmiedeberg's view. From subsequent work on avian erythrocyte nuclei Ackermann concluded that these contained histone and nucleic acid and little else. As a result of these investigations the idea gradually developed that the cell nucleus was almost entirely composed of nucleic acid and either protamine or histone.

It was soon realized that histone was a more complex protein than the protamines and was distinguished from them by its less basic properties and by its tyrosine content. (Protamines generally contain no tyrosine.) Its basic properties are due to the high ratio of basic to acidic amino acids and in particular to its arginine content. Early analyses showed this to be about 10%. Kossel, who discovered histone in the nuclei of bird erythrocytes, turned his attention to the sperm of certain fish in which histone replaced protamine. He found them to correspond generally to thymus histone in their basic amino acid content (see Table 1).

A full account of the early work on the protamines and histones is given in Kossel's (1928) monograph.

In early investigations on the nucleic acids and nucleoproteins the sources of material (i.e., sperm, avian erythrocytes, calf thymus and yeast) were rich either in deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). This undoubtedly played a part in leading to the erroneous conclusions on the occurrence of the nucleic acids within the

plant and animal kingdoms and their location within the cell.

Until 1924, and indeed in many quarters until later, it was thought that DNA was confined to the animal cell and that RNA replaced it in the plant cell. In 1924, however, Feulgen and Rossenbeck demonstrated the presence of DNA in the wheat embryo nucleus and suggested that RNA was a constituent of the cytoplasm. The presence of DNA in plant tissues was also shown by Boas and Biechele (1932), by Kiesel and Belozerski (1934) and by others. DNA was isolated from rye embryo nuclei by Feulgen, Behrens and Mahdihassan (1937), and proof of the presence of RNA in the cytoplasm was obtained the following year (Behrens 1938).

About the same time Jorpes (1934) working with the -nucleoprotein of pancreas, originally prepared by Hammarsten in 1894, showed it to contain a pentose nucleic acid. The occurrence of RNA in animal tissues had been suggested by earlier investigations but was now conclusively demonstrated and has been confirmed by later work.

Thus, about 1940, the old conception of RNA as "plant nucleic acid" and of DNA as "animal nucleic acid" was being replaced by the view that DNA was a nuclear constituent of both plant and animal cells while RNA occurred in the cytoplasm.

Since the time of Miescher and Kossel little concentrated work was done on the chemistry of the cell nucleus until a few years ago. Even the materials used by the early

workers, i.e., sperm and bird erythrocytes, received little attention although several investigators carried out experiments on the oxygen consumption of isolated erythrocyte nuclei (Warburg, 1910; Miyake, 1933; Yakusizi, 1933 a b; Laskowski, 1942; Dounce and Lan, 1943; and Hunter and Baufield, 1943).

1.2. The Isolation of Cell Nuclei.

The main obstacle to investigations of the composition of cell nuclei has been the lack of a general method of preparation. Behrens (1932) developed a method, using calf heart muscle, whereby the powdered frozen-dried tissue was allowed to settle in cylinders of organic solvents of increasing density. Later (Feulgen et al 1937) the method was applied to plant cells and DNA isolated from rye embryo nuclei. The procedure is, however, tedious and has been little used.

In 1937 Crossman noted that nuclei were liberated from cardiac muscle which had been teased in 5% citric acid. This observation was adapted to the preparation of nuclei in bulk by Stoneburg (1939) who immersed the minced tissues in 5% citric acid, centrifuged off the nuclei and dissolved the contaminating muscle debris in a solution of pepsin in hydrochloric acid. Nuclei of beef heart muscle, rabbit thigh muscle, tumour and pus cells were prepared in this

manner but the method was not successful when applied to thymus and liver. Marshak (1940 and 1941) like Stoneburg employed 5% citric acid but without the use of pepsin-hydrochloric acid solution. Nuclei were isolated from liver and tumour.

An extensive investigation of methods for the preparation of nuclei using citric acid has been made by Dounce (1943 a, b and c). He found that a pH value of 3.8-4.0 was most suitable for preparing liver nuclei although pH 6.0-6.2 was satisfactory when enzyme studies were envisaged. Above pH 6.5 the nuclei tended to disintegrate while pH values between 4.0 and 5.9 caused cytoplasmic granules to agglutinate thus making the isolation of nuclei impossible. At a pH of about 3.0 or less it would be expected that some histone might be extracted during the isolation, but Dounce (1943c) has found such a high acidity necessary for the isolation of tumour nuclei. He also obtained evidence for a loss of nucleoprotein when the preparation was carried out at pH 6.0-6.2.

The procedure adopted by Dounce is to homogenize the tissue in a Waring blendor in an ice cold solution of citric acid at the required pH and to spin down the nuclei initially at fairly high speed. Cytoplasmic contamination is subsequently removed by suspending the sediment in ice-cold water several times and centrifuging each time at a relative-

ly low speed so as to bring down the nuclei leaving cytoplasmic material in the supernatant fluid. When necessary the suspensions may be brought to the required pH by addition of citric acid as the pH may rise during the isolation.

Methods which have subsequently been used are in general modifications of the Dounce procedure. For example, Mirsky and Pollister (1946) homogenized the tissues in 1% citric acid and used 0.2% citric acid for washing (see p. 29).

Somewhat different methods for preparing nuclei have been employed on occasion recently. Nash (1949) used 0.85% NaCl containing 2 ml. 0.1 N NaOH/l in the initial homogenising of mouse liver (final pH 6.8-7.2). The nuclei were then washed with 2% citric acid. Arnesen, Goldsmith and Dulaney (1949) have employed 8.5% (isotonic) sucrose solution $\frac{M}{125}$ with respect to citric acid, using sucrose solution (without citric acid) for subsequent washings of spleen nuclei from normal and leukaemic mice. Mouse spleen nuclei have also been prepared using a sucrose solution (0.88 M) containing $CaCl_2$ (0.002 M) by Petermann and Schneider (1950). Sucrose solutions of different densities have been used in a novel procedure developed by Wilbur and Anderson (1951).

Bird erythrocyte nuclei may be easily prepared from red cells washed in physiological saline. These can be

laked by freezing and thawing (Warburg, 1910), with lysolecithin (Laskowski, 1942), saponin (Dounce and Lan, 1943), or tyrothricin (Villela, 1947) and the nuclei washed with physiological saline until the haemoglobin has been removed. Laskowski and Ryerson (1943) showed that erythrocyte nuclei passed into solution in sodium chloride solutions of concentrations greater or less than 1%.

Sperm nuclei were early prepared by suspending the sperm in slightly acid media and centrifuging down the sperm heads, the body of the sperm having been dissolved. Recently Zittle and O'Dell (1941) obtained sperm heads by ultrasonic treatment of whole bull sperm. The heads comprised 51% of the dry weight of whole sperm and gave the analysis shown in Table 2. Available data on the composition of sperm and sperm heads is given in Table 3.

1.3. The Lipids of the Cell Nucleus.

The first investigation of nuclei prepared in citric acid, that of Stoneburg (1939), was concerned with the lipids. The only other studies relating primarily to nuclear lipids are those of Haven and Levy (1942), Williams, Kaucher, Richards and Moyer (1945) who made a fairly exhaustive investigation of whole liver and liver nuclei, and Tyrrell and Richter (1951) who have studied the nuclei of human brain cortex. Some of the results of the above groups are given in Table 4.

Table 2.

Per cent Composition of dry lipid-free bull sperm heads.
(Zittle & O'Dell, 1941).

Lipid	7.0	Nitrogen	18.5
Phosphorus	4.0	Sulphur	1.6
DNA	40.4	Cystine Sulphur	1.07
DNA (by diphenyl-amine reaction)	40.5	Ash	2.1

Table 3.

The Composition of Sperm and Sperm Heads (% dry weight).

Species	Material	Phosphorus	Sulphur	Nitrogen	Reference
Bull	Sperm	2.3	1.2		Miescher (1897)
"	"	2.7	1.6	16.4	Zittle & O'Dell (1941)
"	" heads	4.7	1.7		Miescher (1897)
"	" "	4.0	1.6	18.5	Zittle & O'Dell (1941)
Guinea-pig	Sperm	1.3	1.7		"
Human	"	1.5-4.2	1.2		"
Herring	"	6.1		21.1	Mathews (1897)
"	"	6.4		20.8	Steudel (1913)
"	" heads	4.1-5.9			Stedman & Stedman (1947)
White fish	" "	6.3		21.4	Lynch (1920)
Salmon	" "	5.4-6.3			Stedman & Stedman (1947)
Trout	Sperm	5.0		18.3	Pollister & Mirsky (1946)
Cod	" heads	2.8			Stedman & Stedman (1943a)

Table 4.

Concentration of lipids in isolated cell nuclei (% dry weight).

	Total lipid	Neutral fat	Neutral fat fatty acids	Total Cholesterol	Phospho lipid	Reference
Beef heart muscle			6.5	3.6	15.7	Stoneburg (1939)
Rabbit thigh muscle			1.8	3.6	3.0	"
Pus cells			26.0	2.5	12.0	"
Rat carcino-sarcoma 256			18.0	4.6	7.5	"
"					9.7	Haven & Levy (1942)
Dog liver	16.5	4.6		1.2	10.5	Williams et al (1945)
Rat liver	18.1	4.2		1.5	12.5	"
Rat liver tumour	14.7	4.4		2.7	7.3	"
Rat liver	3.2-10.8					Dounce (1943b)
" "	14.0					Villela & Ubatuba (1948)
Fowl Erythrocytes	14.0					Dounce & Lan (1943)
" "					4.0	Jeener (1946)
Snake Erythrocytes	12.7					Villela (1947)
Human Brain Cortex				4.1	13.0	Tyrrell & Richter (1951)

From the choline content of the phospholipid fraction of the nuclei of carcino sarcoma 256 Haven and Levy (1942) deduced that it was composed of 40% cephalin and 60% lecithin, with little or no sphingomyelin. Williams et al (1945) obtained similar results for their liver and hepatoma nuclei. There was little difference between the lipid composition of the nuclei and whole tissue. The content of lipids in brain nuclei was generally less than in whole tissue although Tyrrell and Richter (1951) found twice as much cerebroside. An appreciable amount of sphingomyelin was also detected in the nuclei.

1.4. The Nuclear Proteins*.

Early investigations of the proteins of the cell nucleus were confined to three tissues (sperm, avian erythrocytes and thymus) and mainly to sperm, a specialised tissue. It has already been mentioned (p.4) that on the early results a whole system of the protein chemistry of the cell nucleus has been built up to the effect that the only protein present of any consequence is either protamine or histone.

The first indication that the situation was more complex came from the work of Caspersson (1941) who, on the basis of spectrophotometric evidence, concluded that the

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* For a review article see Hamer (1951a).

nucleus contained a higher protein of the globulin type. In 1942 Mayer and Gulick showed that calf thymus nuclei prepared by the Behren's technique contained nucleic acid and histone to the extent of only 60%. The remaining material, which contained a large amount of labile sulphur, consisted in part of a protein of the globulin type of high sulphur content and a fraction low in sulphur.

In 1943 Stedman and Stedman (1943a) announced the discovery of a protein of the cell nucleus which they called chromosomin and which they claimed to be an essential component of the chromosomes. The chromosomin of cod sperm received most attention and was shown to have predominantly acid properties ascribed mainly to the presence of large amounts of glutamic acid. In contrast to histone chromosomin contained a relatively large amount of tryptophan while histone contains very little or none.

Chromosomin was also obtained in large amounts from the nuclei of ox spleen and Walker rat carcinoma. Further studies made by Stedman and Stedman (1943 b and 1947) on the distribution of chromosomin in cell nuclei are summarised in Table 5. From the low histone content of nuclei from growing tissues it was suggested that it may play a part in regulating cell division, the presence of large amounts of histone inhibiting mitosis in resting tissues.

The view of the Stedmans is therefore that the cell

Table 5.

The Composition of Cell Nuclei (Stedman & Stedman, 1943 a,b; 1947)

(% Dry weight, lipid free)

Source of nuclei	Nucleic acid	Histone (as sulphate)	Chromosomin
Cod sperm	12	28	60
Herring sperm	50	18	30
Salmon sperm	59	Similar to herring sperm	
Fowl erythrocytes	43	24	33
Calf thymus	41	25	34
Human thymus	37	25	38
Ox spleen	34	16	50
Mouse spleen	37	28	35
Ox liver	30	24	46
Mouse liver	30	23	47
Human liver	27	17	66
Walker rat carcinoma	26	1.6	72
Mouse carcinoma 2146	32	3	65
Chick embryo	35	3	62.

nucleus is composed of three main components, DNA, histone (or protamine) and chromosomin, of which the last is quantitatively the most important.

Jeener (1946, 1947) has more recently separated a solution of hen erythrocyte nuclei into a protein fraction (33-40% of the total protein) containing about 90% of the total phospholipid and richer in tryptophan (0.5%) than the second fraction which is essentially DNA-histone. The non-histone protein would appear to resemble Stedman's chromosomin.

Of greater significance is the recent work of Mirsky and Pollister (1946) on the nucleoproteins of the cell nucleus to which they have given the name "chromosins". These complexes have been isolated by methods developed earlier (Mirsky and Pollister 1942, 1943) for the preparation of nucleohistones and depend on the solubility of the complexes in 1 M NaCl and their insolubility in 0.14 M NaCl.

"Chromosins" were prepared from a variety of mammalian tissues and from avian erythrocytes, fish liver and erythrocytes, wheat germ, and type III Pneumococci. The complexes could be prepared from isolated nuclei or from whole tissue homogenates from which cytoplasmic material had been washed out with 0.14 M NaCl. They were soluble in M NaCl from which they were precipitated by dilution with

water and contained large amounts of DNA and histone, a little RNA and variable quantities of a second protein which was shown to contain about 1% tryptophan. This non-histone protein may well correspond to Stedman's chromosomin. However it was not observed in the chromosin of trout sperm (Pollister and Mirsky, 1946).

The tryptophan-containing protein of Mirsky and Pollister has also been prepared from what has been claimed as isolated chromosomes (Mirsky and Ris 1947 a and b), i.e., the nuclear fragments released from resting cells by vigorous mechanical treatment of the tissue in physiological saline followed by differential centrifugation. The greater part of the material is soluble in 1 M NaCl and consists essentially of DNA and histone. The small amount of material insoluble in M NaCl (the "residual chromosomes") retains the original chromosome-like structure and can be centrifuged out at high speeds. It consists almost entirely of a tryptophan-containing protein and RNA. In thymus chromosomes the residual material accounted for 8% of the whole chromosomes (which contained 37% DNA and 55% histone) and was shown to contain about 10% RNA and 2% DNA. The residual protein contained 1.36% tryptophan while the histone, which the authors obtained by extraction with dilute hydrochloric acid, contained 0.14% tryptophan. Mirsky, however, has suggested

that two histones are present in the nuclear material of thymus. More conclusive evidence for this has recently been presented by Stedman and Stedman (1950).

Further work by Mirsky (1947) has shown that in cells having little cytoplasm (thymus lymphocytes, trout sperm and fowl erythrocytes) the "residual chromosome" makes up a small proportion of the total "chromosome" but in liver, kidney and salivary gland, in which there is abundant cytoplasm, the proportion is very much greater.

Lamb (1949, 1950) has criticised Mirsky's work on the grounds that the threads of nuclear material, claimed to be isolated chromosomes, are in fact fragments of nuclei which have been drawn into strands of chromatin by the homogenising technique. However there seems to be little doubt that the "isolated chromosomes" of Mirsky are largely nuclear in origin although the presence of cytoplasmic material cannot be excluded. It is also probable that some of the material of the "isolated chromosomes" is dissolved out in the 0.14 M NaCl used in their preparation.

Recent work of Wang, Kirkham, Dallam and Mayer (1950) (see also Zbarski and Debov, 1948) suggests the presence of a third protein in the cell nucleus. Wang et al, working with rat liver nuclei, calf thymus "chromosomes" and boar sperm heads, have observed a protein fraction in the residue

which is insoluble in M NaCl which is not extracted by dilute acid or dilute alkali. The protein extracted from the M NaCl residue with dilute alkali may correspond to Stedman's chromosomin.

The amino acid composition of the nuclear proteins is presented in Tables 6, 7, 8 and 9.

1.5. Enzymes in the Cell Nucleus.*

The first enzyme studies of isolated nuclei were those of Behrens (1939) on liver, who showed arginase to be distributed equally between the cytoplasm and nucleus. The lipase activity of the nuclei was only 5% of that of the cytoplasm. Behrens did not pursue these studies further but an extensive investigation of the enzymic capabilities of the rat liver cell nucleus has been made by Dounce and his associates. Their results are summarized in Table 10. Observations on the enzymes in the nuclei of other tissues are given in Table 11.

The high activity of alkaline phosphatase observed in the nuclei of rat liver and brain cortex is of interest especially as it is abundant in the chromosomes (Willmer, 1942; Wachstein, 1945) and Danielli and Catcheside (1945) have observed that it is restricted to the Feulgen positive bands of salivary gland chromosomes. That the enzyme is present in "isolated chromosomes" has been shown by Mirsky

* For review articles see Dounce (1950 a and b) and Bradfield (1950).

Table 6.

The Amino Acid Composition of the Protamines: Salmin

(Amino acid N as % total N)

	Taylor (1908-09)	Kossel (1928)	Tristram (1947)	Block et al (1945 & 1949)	Hamer & Wood-house (1949)
Arginine	91.7	87.4	85.2	88.4	91.0
Alanine			1.1	1.5	0.3
Glycine			2.9	3.3	2.2
Isoleucine			1.6	1.2	0.6
Serine	8.7	7.8	9.1	7.0	2.5
Proline	10.8	11.0	5.8	8.3	2.0
Valine					1.5

Table 7.

The Amino Acid Composition of the Protamines: Clupein.

(Amino acid N as % total N)

	Kossel (1928)	Block et al (1949)	Felix et al (1950)	Waldschmidt-Leitz et al (1951)
Arginine	88.0	87.1	89.7	+
Alanine		4.7	1.89	+
Isoleucine		1.0	0.43	0
Valine			1.60	+
Serine		3.4	2.42	+
Threonine		1.9	0.65	0
Proline		8.2	2.22	+

Table 8.

The Amino Acid Composition of Histones

(see also Daly, Mirsky & Ris, 1951)

(amino acid N as % total N).

	1	2	3a	3b	4	5
Alanine		+	+	+	6.0	16.5*
Glycine		-	+	+	5.2	6.0
Leucine		+	+	+	3.05	10.1
Isoleucine		+	+	+	12.0	
Valine		+	+	+	4.9	3.98
Serine		+	+	+	3.45	4.6
Threonine		+	+	+	3.1	5.4
Arginine	+	+	+	++	30.7	23.4
Histidine	?	-	+	+	4.0	1.63
Lysine		+	++	+	10.8	11.75
Aspartic acid		+			3.3	4.9
Glutamic acid		+			2.25	16.5†
Phenylalanine		+			1.9	1.66
Tyrosine	+	+			1.4	1.1
Tryptophan	+	?				
Proline		+	+	+	2.7	2.19
Ammonia					4.8	7.25
Unidentified		1				

* Including glutamic acid

† Including alanine.

1. Sperm of Selachians. Cardoso & Pirro (1947).

2. Calf thymus, rat liver and fowl erythrocytes. Davidson & Lawrie (1948).

3. Sperm of (a) sea urchin and (b) mollusc. Hultin & Herne (1949).

4. Calf thymus. Hamer (1951).

5. Rat liver. Analysis (1 sample) carried out by Dr. G. Leaf of this Department.

Table 9.

The Amino Acid Composition of Non-histone Proteins

(Amino acids as % total protein).

	1	2	3	4	5	6	7	8
Alanine			+		+	+	+	+
Glycine			+		+	+	+	+
Leucine			+	3.8	+	+	+	+
Isoleucine				2.6	-		+	-
Valine			+	2.5	+	+	+	+
Serine			+		+	+	+	-
Threonine			-	1.9	+	+	+	-
Cystine	0.4	+	-		-	+	-	-
Methionine			-	0.9	-	+	-	-
Arginine	9.5	+	+	5.2	+	ca.10	+	+
Histidine	5.0		-	0.9	-	+	?	-
Lysine	11.0		-	4.4	+	+	+	+
Aspartic acid	+		+		+	+	+	+
Glutamic acid	+		+		+	+	+	+
Phenylalanine			+	1.2	+	+	+	-
Tyrosine		+	+	1.7	-	+	+	-
Tryptophan	+	1.36	0.76 1.20	0.2	-	+		-
Proline			+		-	+	+	+
Unidentified			2		3	2		

1. Cod sperm chromosomin. Stedman & Stedman (1943a).
2. Residual chromosomes (thymus). Mirsky & Ris (1947).
3. Non-histone protein of calf thymus, rat liver and fowl erythrocyte nuclei (figures refer to thymus and liver respectively). Davidson & Lawrie (1948).
4. Whole fowl erythrocyte nuclei. Melampy (1948).
5. Carp erythrocyte chromatin. Yasuzumi & Miyao (1950).
6. Boar sperm: fraction precipitated at pH6 from N NaOH extract. Thomas & Mayer (1949).
7. Nuclear sap (Newt oocyte). Brown, Callan & Leaf (1950).
8. Salivary gland chromosomes. Kirby (1948).

Table 10.

Enzymes in the Rat Liver Cell Nucleus.

Enzyme	Activity in nucleus as % activity in whole tissue		Reference
Aldolase	40		Dounce & Beyer (1948a)
-Amino acid oxidase	100		Lan (1943 & 1944)
Arginase*	113		Dounce (1943)
			" & Beyer (1948b)
Catalase	50		Dounce (1943 & 1950)
Choline oxidase	0		Lan (1943 & 1944)
Cytochrome oxidase	55		Dounce (1943a)
Cytochrome C	low		Dounce (1943b)
Enolase	50		Dounce (1950)
Esterase	50		Dounce (1943a)
Alkaline phosphatase	192		"
Acid phosphatase	25		"
Phosphorylase	66	(after grinding)	Dounce (1950)
	26	(without grinding)	"
Lactic dehydrogenase	40		Dounce (1943a)
Succinic dehydrogenase	0		"
Uricase	100		Lan (1943 & 1944)

* Increased in regenerating liver nuclei. Dounce & Beyer (1948a).

Very low in rat liver nuclei. Euler, Fischer, Hasselquist & Jaarma (1945).

Note: Xanthine oxidase, l-amino acid oxidase and l-proline oxidase were not detected in the nuclei of rat liver (or pig kidney) by Lang & Siebert (1950).

Table 11.

Enzymes in the Cell Nucleus.

Enzyme	Activity in nucleus as % activity in whole tissue	Reference
Frog oocytes		
Arginase	Traces in nucleus & cytoplasm	Brachet (1942 & 1943)
Ribonuclease	ca. 100	"
Alkaline phosphatase	ca. 100	"
Bull sperm		
Cytochrome oxidase	4.8	Zittle & Zitin
Cytochrome C	Low in sperm head	" (1942a)
Fowl erythrocytes		
Acid phosphatase	100 (activity low)	Dounce & Seibel (1943)
Human cerebral cortex		
Alkaline phosphatase	294	Richter & Hultin
Acid phosphatase	101	" (1951)
Acetyl choline esterase	132	"
Carbonic anhydrase	33	"
Jensen sarcoma		
Catalase	Very low in nucleus	Euler, Fischer et al. (1945)
Rat kidney		
Aldolase	40	Dounce (1950b)
Arginase	0	"
Catalase	50-80	"
Ox pancreas		
Esterase	50	"
Lipase	50	"

(1947), who noted that it was confined to the "residual chromosomes". A similar observation has been made by Jeener (1946) who showed that treatment of cell nuclei with 0.6 M KCl gives an insoluble residue rich in phosphatase.

The possible relationship between alkaline phosphatase and the metabolism of DNA was suggested by the work of Brues, Tracy and Cohn (1944). It was shown that alkaline phosphatase and the rate of DNA turnover was increased in the nuclei of rat liver after partial hepatectomy. This has received support from the observations of Brachet and Jeener (1946) on the correspondence between the alkaline phosphatase activity in the nuclei of several rat tissues and the DNA turnover rate of the same tissues (Hevesy and Ottesen 1943).

From the work of Dounce it is clear that the cell nucleus has a not inconsiderable enzymic equipment. It is even possible that this may be qualitatively and quantitatively greater than the tables show since some material is lost during isolation of the nuclei. On the other hand it is possible that in some cases high enzyme activity may be due to adsorption of cytoplasmic enzymes. Such contamination is considered unlikely by Dounce and Beyer (1948 b) and Dounce (1950 a).

There is little point in investigating the content of small molecular-weight compounds (e.g., coenzymes) in

nuclei obtained by the citric acid method since heavy losses are bound to occur during preparation, but such compounds may be estimated in nuclei isolated by the Behrens technique provided that they are insoluble in lipid solvents. Isbell, Mitchell, Taylor and Williams (1942) have investigated the B vitamins by this method, with the results shown in Table 12.

With the exceptions of pyridoxin, biotin and inositol the concentration of the vitamins is about threefold greater in beef heart nuclei than in whole tissue. There is little difference between the concentrations in the whole tissue and nuclei of mouse cancer. Villela (1947) has shown that isolated nuclei of snake erythrocytes contain 80 and 120 $\mu g/g$. respectively of thiamin and nicotinamide. Because of the method of preparation of the nuclei these are no doubt minimal values.

The Behrens technique has also been used by Williams and Gulick (1942) to examine the inorganic constituents of the cell nucleus with the results shown in Table 13. Other data obtained from nuclei prepared in aqueous media are included.

1.6. The Nucleic Acid Content of the Nucleus.

Deoxyribonucleic acid is found exclusively in the nucleus, usually in quite large amounts, and is intimately associated with the chromosomes according to conventional

Table 12.

B Vitamins in the Cell Nucleus. (Isbell et al, 1942)

(μg. vitamin per g. dry material).

Vitamin	Whole Beef Heart	Beef Heart Nuclei	Mouse Cancer	Mouse Cancer Nuclei
Thiamin	32	90	9.0	7.4
Riboflavin	34	130	8.3	7.0
Nicotinic acid	320	900	130	95
Pyridoxin	4.4	4.2	0.87	0.90
Pantothenic acid	75	270	60	43
Biotin	0.52	0.25	0.35	0.27
Inositol	7600	2000	450	400
Folic acid	1.1	3.9	17	13

Table 13.

The Inorganic Constituents of the Cell Nucleus.

Metal	Source of nuclei	% dry wt.	Reference
Ca	Calf thymus	1.35*	Williams & Gulick (1942)
	Rat liver	2.3×10^{-4}	Dounce & Beyer (1948b)
	White fish sperm	9.7×10^{-2}	Lynch (1920)
Mg	Calf thymus	8.5×10^{-2} †	Williams & Gulick (1942)
	Rat liver	7.0×10^{-3}	Dounce & Beyer (1948b)
Fe	Rat liver	1.1×10^{-2}	Dounce & Beyer (1948b)
	Bull sperm	4.0×10^{-3}	Zittle & Zitin (1942b)
	White fish sperm	nil	Lynch (1920)
Cu	Rat liver	2.5×10^{-2}	Dounce & Beyer (1948b)
Al	"	1.7×10^{-2}	"
Mn	"	1.4×10^{-3}	"
Zn	"	1.2×10^{-3}	"

* Whole tissue 7.4×10^{-1}

† Whole tissue 3.0×10^{-2}

genetic theory. It has therefore received much attention in studies of isolated cell nuclei. Until recently it has been customary to express the DNA content of the nucleus as a percentage of the dry weight of nuclei in bulk (see Table 14). As the method of isolation influences the overall composition of nuclei isolated in bulk (Dounce 1943 a) variable results have been obtained by different workers (Table 14). This is apparently due mainly to loss of protein. A fuller account of this question will be presented in the discussion.

Recent cytochemical investigations suggest that RNA is also present in the nucleus. Caspersson was the first to recognise the importance of the microspectrographic method for the study of nucleic acids in the cell (see Caspersson 1947 and 1950) and his researches have done much to focus attention on the genetic and metabolic implications of the nucleic acids. Caspersson and Schultz (1940) showed that the nucleolus absorbed ultraviolet light strongly and since it gave a negative Feulgen reaction for DNA the presence of RNA was presumed. Evidence in support of this came from the work of Brachet (1940), who used staining and enzymic techniques, and of Davidson and Waymouth (1946) who showed that the nucleolus did not absorb ultraviolet light after treatment with ribonuclease.

Table 14.

The DNA content of isolated cell nuclei.

Source of nuclei	pH used in isolation	% dry wt.	Reference
Fowl erythrocytes	6.8-7.0	37.8-38.1	Dounce (1943c)
"		43	Stedman & Stedman (1943b)
Snake erythrocytes		31 - 64	Villela (1947)
Calf thymus		44	Stedman & Stedman (1943b)
"		37.8	" (1947)
"		ca. 28	v.Euler, Hahn, Hasselquist, Jaarma & Lundin (1945)
Human thymus		37	Stedman & Stedman (1947)
Ox spleen		34	" (1943a)
Mouse spleen		35.8-38.0	" (1947)
Calf heart muscle		25	Behrens (1932)
Frog intestine		25.9	Brachet (1941)
Rat liver	3.8-4.0	19.7-23.9	Dounce (1943b)
"	6.0-6.2	12.8-20.2	" (1943b)
"	3.0	25.3	" (1943c)
"	2.4	38.3-40.3	" (1943c)
"		30	Villela & Ubatuba (1948)
"	6.2	21.6-28.9	Catchpole & Gersh (1950)
Mouse liver		28.9-30.7	Stedman & Stedman (1947)
Ox liver		29.2-30.4	" (1947)
Human liver		27.4	" (1947)
Walker rat carcinoma	3.0	21.0-21.6	Dounce (1943c)
"		26	Stedman & Stedman (1943b)
Hepatoma 31	2.4	15.2-25.3	Dounce (1943c)
Hepatoma		9.8-24.6	Catchpole & Gersh (1950)
Rat Jensen sarcoma		ca.28	v.Euler, Fischer, Hasselquist & Jaarma (1945)
Mouse carcinoma 2146		32	Stedman & Stedman (1943b)
Chick embryo		35	" (1943b)

Evidence has also been presented which indicates the presence of RNA in the chromosomes (see Caspersson 1947; Brachet 1942; Mirsky 1947). Kaufmann, McDonald and Gay (1948) and Kaufmann (1949) believe that RNA is present in the chromosomes in two forms, "constant" and "labile".

Few results are avilable for the RNA content of the nucleus and these have been obtained only by estimating pentose in isolated cell nuclei. Early results of Brachet (1941 a) indicate that about 3% RNA (dry weight) is present in the nuclei of frog intestine, the ratio of RNA to DNA being 0.11. A figure of 0.2 is given by Davidson and Waymouth (1943) for embryo sheep liver nuclei and 0.25 for rat liver nuclei by Bergstrand, Eliasson, Hammarsten, Norberg, Reichard and Ubisch (1948). Vendrely and Vendrely (1948) examined various tissues of the ox and found the ratio of RNA to DNA in the nuclei to vary from 0.05 to 0.2.

The conclusion that RNA is present in the cell nucleus is therefore drawn from a few cytochemical observations and the detection of pentose (or a component reacting like pentose) in isolated cell nuclei. The evidence is strongly suggestive but cannot form a rigid proof.

The close association of the amount of DNA in the nucleus with cellular genetics has been emphasized by the preliminary work of Boivin, Vendrely and Vendrely (1948)

and Vendrely and Vendrely (1948) who showed that the nuclei of somatic cells of the different tissues of a given animal species contain the same amount of DNA per nucleus, whereas the sperm cells contain half this value; and also that this constancy prevails throughout several mammalian species. If we presume that DNA is a fundamental constituent of the chromosomes these results are in agreement with current genetic theory and are significant from the standpoint of evolution (see Mirsky and Ris, 1951).

The relationship between the DNA content of the nucleus and cell division has been considered by Ris (1947) who showed that in mitosis in onion root tips the amount of DNA was doubled in the nucleus during prophase. Brachet (1941 b) had observed the DNA content of chromosomes to be increased at metaphase. Ris (1947) studying meiosis in the grasshopper observed no increase in DNA during the second division so that an early spermatid contained 25% of the nucleic acid of the first spermatocyte at metaphase. These observations of Ris support the findings of Vendrely and Vendrely (1948). But Pasteel and Lison (1950), using cytochemical techniques, indicate that the DNA content of resting cells is less than that corresponding to the diploid value and that this is reached in dividing cells at or before prophase. After division of the chromosomes the daughter nuclei contain half the prophase complement of DNA

so that DNA synthesis must also take place after nuclear division.

1.7. The Biological Activity of the Nucleic Acids of the Nucleus. *

The metabolic activity of the nucleic acids has recently been studied with the aid of isotopes. Using ^{32}P Hahn and Hevesy (1940) showed the turnover of DNA phosphorus to be very low in the organs of the rabbit and to vary considerably from organ to organ (Hevesy and Ottesen, 1943; Ahlström, Euler and Hevesy, 1945) (see Table 15).

In resting tissues the turnover of DNA phosphorus is much slower than that of the RNA of the same tissue (Hammarsten and Hevesy 1946; Davidson 1947). It would appear that the DNA bases are also metabolically inactive since Davidson and Raymond (1947) showed that ^{15}N labelled ammonia was incorporated into the DNA of pigeon tissues to a very small extent. Brown, Petermann and Furst (1948) using ^{15}N labelled adenine obtained similar results when they showed that the incorporation of ^{15}N into the DNA purines of rat viscera was only 3.5% of that for RNA purines.

Since DNA must be synthesised during cell division one might expect the turnover of DNA to be increased in growing tissues. Relatively high turnover rates have been observed for the DNA of the tissues of young rats by Ahlström,

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* See Hevesy (1948), Brown (1950) and Davidson (1950).

Table 15.

Turnover of DNA in rat tissues
(Hevesy & Ottesen, 1943).

Tissue	Daily renewal (%)
Intestinal mucosa	15.0
Spleen	5.8
Testes	2.6
Muscle	1.9
Liver	1.0
Kidney	0.6
Brain	0.6

Euler and Hevesy (1944) and Andreasen and Ottesen (1945) and it has been found that the incorporation of ^{32}P into the DNA of regenerating liver and hepatoma is more rapid than into that of the resting tissue (Brues, Tracy and Cohn, 1944). Bergstrand et al (1948) similarly demonstrated an increased incorporation of glycine labelled with ^{15}N into the DNA purines of regenerating rat liver. The low activity of DNA from non-growing tissues and higher activity in tissues showing a high mitotic rate suggest that DNA turnover occurs only in dividing cells. However, evidence against this view is provided by Euler and Hevesy (1942) and Ahlström et al (1944) who showed that in rapidly growing tissues only about half the labelled DNA could be accounted for as an absolute DNA increase.

Exposure to X-rays brings about a great reduction in DNA turnover in both resting and growing tissues. Ahlström et al (1944) observed a decrease in the incorporation of ^{32}P into the DNA of the tissues of young and adult rats exposed to X-rays and Hevesy (1948) working with bird erythrocytes observed a similar phenomenon in the fowl. This effect of X-rays was also observed with tumour tissue by Euler and Hevesy (1942) in a study of Jensen sarcoma. In the opinion of Hevesy (1948) it is the rate of DNA synthesis which is affected, alteration in DNA breakdown playing little or no part.

The original work on isolated cell nuclei obtained from animals receiving ^{32}P is that of Marshak (1940, 1941) who showed a rapid uptake of ^{32}P by liver and lymphoma nuclei and by the nuclei of regenerating liver. The lymphoma nuclei showed a much higher ^{32}P content than those of liver and to retain this over a long period. These results suggest the presence in the nucleus of phosphorus compounds showing a high turnover rate.

In experiments with ^{32}P a much higher specific activity was found for nucleoprotein phosphorus (acid insoluble non-lipid phosphorus) than for DNA phosphorus in isolated liver nuclei by Hevesy (1947), while Marshak (1947, 1948) observed that the specific activity of phosphorus liberated from nuclei incubated in 0.85% NaCl containing $MgSO_4$ (0.006 M) at 37° with or without ribonuclease was much higher than that released by deoxyribonuclease. Marshak therefore postulated the presence, in the nucleus, of a nucleic acid of high metabolic activity intermediate in type between RNA and DNA and suggested that it may be the precursor of both.

Bergstrand et al (1948) have demonstrated the presence of a highly active nucleic acid more conclusively by showing that the purines of "nuclear RNA" of liver contained about twice as much ^{15}N as those of cytoplasmic RNA after administration to rats of glycine labelled with ^{15}N. The

DNA purines contained little ^{15}N. In regenerating liver all fractions showed an enhanced ^{15}N content, especially DNA. These results are given in detail in Table 16.

The occurrence of a highly active RNA in the nucleus would be of great significance in the light of the views of Caspersson (1950) and of Brachet (1950) on the relationship between RNA and protein synthesis.

The cell nucleus has, in the past, been largely the domain of the geneticist and cytologist. However increasing attention given to it by bio- and cyto-chemist has revealed that it consists, in large part, of three constituents, DNA, a basic and an acidic protein. Indications that it plays a much wider role in life processes than those defined by genetic consideration are revealed in recent studies of its enzymic content and by isotope investigations.

It is the purpose of the present investigation to study further the composition of the cell nucleus and its constituents, particularly the phosphorus-containg compounds, and to advance our knowledge of the biological activity of these with the aid of isotopes. Special consideration will be given to the "RNA" and "phosphoprotein" of the nucleus.

In order to compare nuclei from different sources in a more logical manner than hitherto it is intended to ex-

Table 16.

Incorporation of ^{15}N into the nucleic acids of rat liver. (Bergstrand et al, 1948)

(Results expressed as atom per cent excess ^{15}N.)

		Normal liver	Regenerating liver
Cytoplasm RNA	Guanine	0.09	0.97
	Adenine	0.04	0.43
	Uridine	0.21	0.55
Nuclei RNA	Guanine	0.16	1.35
	Adenine	0.12	0.67
DNA	Guanine	0.03	0.68
	Adenine	0.02	0.33.

press the results of analyses in terms of the number of nuclei analysed (i.e., as content per nucleus), as well as in more conventional ways.

Section 2. Experimental Methods.

2.1. Animals.

The following experimental animals have been used in the present investigations:-

(a) Fowls. Brown Leghorn fowls were obtained from the Poultry Research Centre, Edinburgh.

Fowls carrying GRCH 15 Tumours. The GRCH 15 tumour is a non-filterable fowl sarcoma first produced by the action of 1:2:5:6-dibenzanthracene by Dr. P.R. Peacock of the Glasgow Royal Cancer Hospital and subsequently maintained by serial transplantation (Peacock, 1933).

Transplantation of GRCH 15 Tumour.

About 2 g. of the non-necrotic portions of a tumour from a freshly killed bird was ground in a Craigie (1949) mincer and suspended in about 5 ml. physiological saline. The suspension was filtered through cheese-cloth and 0.5 ml. injected into each pectoral muscle of a young fowl. A tumour of moderate size (50-100 g.) developed within one month. Tumour tissue removed from a decapitated bird was either used immediately or was stored in a deep freeze cabinet until required.

(b) Rabbits. Various strains of rabbit have been used ranging in weight from 1.5 to 3 kg.

(c) Rats. Albino rats, weighing 200-300 g., from the

Departmental Colony were used in most experiments. They were maintained on a stock diet of cubes.

Partial Hepatectomy. Partial hepatectomy was performed under ether anaesthesia by the procedure of Higgins and Anderson (1931).

2.2. Isolation of Nuclei.

(a) General principles. The citric acid method as used by Dounce (1943 a) and Mirsky and Pollister (1946) has been employed in the present experiments. The cell wall is disrupted mechanically in a high speed mixer in a slightly acid medium. The nuclei may then be separated from the lighter cytoplasmic particles by a process of differential centrifugation.

The requisite degree of acidity can be achieved by the use of any weak acid but citric acid has the added advantage of being able to inactivate calcium and magnesium ions. The former tend to agglutinate cytoplasmic particles (Schneider 1946) while the latter activate deoxyribonuclease (McCarty 1946).

According to Dounce (1950 a) the presence of salts is to be avoided and the use of buffers is therefore precluded.

At all stages in the isolation of nuclei the material must be kept cold. In early experiments the whole process

was carried out in the cold room but for the later experiments an International refrigerated centrifuge was available. Later steps in the isolation were checked by microscopical examination.

All tissues were in general used as soon as possible after removal from the animal. When storage was necessary the tissue was kept in a deep freeze cabinet at -10°.

(b) Isolation of Nuclei from calf thymus, rabbit liver and GRCH 15 tumour. (Modified from Mirsky and Pollister, 1946).

Chilled calf thymus gland and GRCH 15 tumour were freed of fatty and connective tissue and cut into small pieces. The livers, perfused with saline at 37°C, were removed from rabbits killed by cervical dislocation and minced with scissors after removal of connective tissue.

100 g. of chilled tissue was homogenised for 6 minutes in 500 ml. ice cold 1% citric acid in a Waring blendor provided with an ice-jacket. One or two drops of octanol were added to cut down foaming. The homogenate was centrifuged at 2,000 r.p.m. for 10 mins. The supernatant fluid was discarded and the precipitate suspended in about 500 ml. 0.2% citric acid and strained through cheese-cloth and then nylon gauze. The subsequent differential centrifugation procedure is given in Table 17.

Table 17.

Differential centrifugation in the isolation of nuclei from calf thymus, GRCH 15 tumour and rabbit liver.

Details of procedure	Centrifugation data	
	Calf thymus & GRCH 15 tumour	Rabbit liver
1. Suspension centrifuged at	1500 r.p.m. 10 mins.	1500 r.p.m. 10 mins.
2. Precipitate suspended in 350 ml. 0.2% citric acid and suspension centrifuged at	1000 r.p.m. 5 mins.	1500 r.p.m. 5 mins.
3. Procedure 2 repeated two or three times		
4. Precipitate suspended in 350 ml. 0.2% citric acid and suspension centrifuged at	1000 r.p.m. 2 mins.	1000 r.p.m. 5 mins.

(c) Isolation of rat liver nuclei.

For rat liver nuclei the method just described was slightly modified in accordance with the procedure of Bergstrand et al (1948). The animals were exsanguinated under ether anaesthesia and the livers perfused with warm saline, removed, freed from non-hepatic tissue and finely minced with scissors. The liver tissue was then homogenised in 2 vols. 0.05 M citric acid for 6 minutes and the homogenate strained through cheese-cloth after the addition of a further three volumes of 0.05 M citric acid. Differential centrifugation was carried out as in Table 18.

The procedure for the isolation of nuclei varies slightly not only according to the tissue used but for individual preparations from the same tissue. Only in this way can an adequate standard of purity be attained.

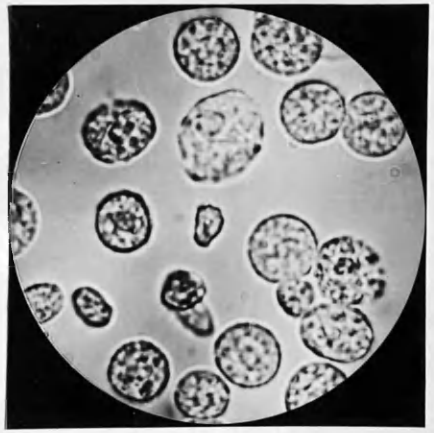
On occasions it was found advantageous to centrifuge a suspension of fairly clean nuclei at high speed for a short time (1-2 mins.) and stir the top buff-coloured layer of precipitate into the supernatant fluid to get rid of cytoplasmic contamination which persists despite the usual treatment. In the later stages of separations purity was invariably checked by microscopic examination. (See Plate 1).

Table 18.

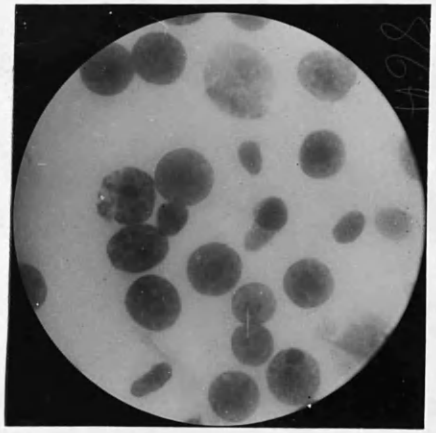
Differential centrifugation in the isolation of rat liver nuclei.

Procedure	Centrifugation data	
1. Homogenate centrifuged at	2000 r.p.m.	10 mins.
2. Precipitate suspended in 5 vols. 0.01 M citric acid and centrifuged at	1500 r.p.m.	10 mins.
3. Precipitate suspended in 4 vols. 0.01 M citric acid and centrifuged at	1500 r.p.m.	5 mins.
4. Precipitate suspended in 4 vols. 0.01 M citric acid and centrifuged at	1250 r.p.m.	5 mins.
5. Procedure 4 repeated 2 or 3 times.		

Plate I. Isolated Rat Liver Nuclei.



(a.) Photograph in visible light by phase contrast. Mag. = 570.



(b.) Photograph in ultraviolet light at 2536 A. Mag. = 490.

(d) Isolation of fowl erythrocyte nuclei
(Method of Dounce and Lan, 1943).

Principle. The defibrinated blood is washed free of plasma with 0.9% NaCl, the erythrocytes laked with saponin and the liberated nuclei washed by centrifugation.

Materials: 0.9% NaCl

0.11 M phosphate buffer, pH 6.8-7.0

0.3 g. saponin in 5 ml. phosphate buffer.

Procedure. The fowls were killed by decapitation. Blood was collected in a beaker from the stump of the neck and whipped to remove fibrin. The blood of fowls bearing GRCH 15 tumour clots very rapidly and it was usually found desirable to homogenise the clots in a cooled Waring blendor in an equal volume of 0.9% NaCl for two minutes and then strain the homogenate through cheese-cloth. The defibrinated blood (or homogenate) was centrifuged, the scum containing white cells removed, and the red cells washed twice with 0.9% NaCl using a volume equal to that of the original blood. The cells were suspended in 0.9% saline so that the final volume equalled that of the blood used and to 100 ml. of the suspension was added 5 ml. 0.11 M phosphate buffer (pH 6.8-7.0) containing 0.3 g. saponin. Laking occurred within 5 minutes. To the laked suspension an equal volume of 0.9% NaCl was added and the nuclei centrifuged at 2500 r.p.m. for 20 minutes. They were then resuspended in saline and

centrifuged as before. The nuclei were washed a further four times and centrifuged ten minutes each time. 2-3 ml. of 0.11 M phosphate buffer were added on each occasion before the nuclei were suspended in saline.

(e) Nuclear counts.

Counting was carried out in a conventional haemocytometer. The nuclei were prepared for counting by suspending them in 0.2% citric acid and noting the final volume. (Fowl erythrocyte nuclei were suspended in 0.9% NaCl). The nuclear suspension was diluted in a blood pipette with 0.2% citric acid (or 0.9% NaCl) so that the suspension used for counting contained about 5×10^6 nuclei per ml. Approximately 500 nuclei were counted on each occasion.

(f) Preparation of dried nuclei.

The counted suspension was centrifuged and the nuclei washed once in ice-cold distilled water. The preparation was transferred to a round-bottomed flask with the minimum of distilled water and the suspension frozen on to the walls in a thin layer by rotating the flask in a mixture of solid carbon dioxide and industrial spirit. The preparation was then vacuum dried, the water being trapped in a condenser cooled in solid carbon dioxide.

The dried nuclei were removed from the flask, weighed and stored in a desiccator.

2.3. Methods of Fractionation.

The methods generally used for the fractionation of samples of tissue and nuclei prior to nucleic acid analysis are based on those of Schmidt and Thannhauser (1945) and Schneider (1945).

The sample was extracted with acid, usually trichloracetic acid, to remove acid soluble compounds including simple nucleotides and lipid was then removed with appropriate lipid solvents (Fig.1).

The residue (nucleoprotein residue) contained protein-bound phosphorus compounds including RNA, DNA and possibly phosphoprotein. This latter when present usually accounts for a very small proportion of the phosphorus of the residue which represents, for most practical purposes, the total nucleic acid phosphorus.

In the Schmidt and Thannhauser procedure the two nucleic acids are separated by incubation of the nucleoprotein residue in N alkali at 37°C to convert RNA to acid soluble mononucleotides leaving DNA still in an acid-insoluble form. The DNA and degraded protein are precipitated with acid, the ribonucleotides remaining in the supernatant fluid along with inorganic phosphate derived from phosphoprotein.

Schneider's method involves extraction of the nucleic acids of both types from the nucleoprotein residue with hot trichloracetic acid and estimation of RNA and DNA

by methods for determining pentose and deoxypentose. The phosphorus not so extracted is supposed to be derived only from phosphoprotein.

a. Details of the procedure of Schmidt and Thannhauser, as it has been used in the present study, are given in Figure 1.

b. The Schneider procedure. In the Schneider procedure acid soluble material and lipids are removed in the same manner as in the method of Schmidt and Thannhauser. The nucleoprotein residue, from 100 mg. dry nuclei, is extracted with 5 ml. 5% TCA at 90°C for 15 mins., cooled, centrifuged and washed with two portions of about 1 ml. each of 5% TCA. The combined supernatant and washings is called the Schneider Extract and the insoluble material is named the Schneider Residue.

c. Fractionation of the Schneider Residue. If all the phosphorus in the Schneider Residue is phosphoprotein phosphorus it should appear as inorganic phosphate on incubation in alkali. The Schneider residue has been investigated using the Schmidt and Thannhauser procedure as shown in Figure 2.

A scheme of analyses carried out on the various fractions of the Schmidt and Thannhauser, and Schneider procedures is shown in Table 19.

d. Methods used in Ionophoresis Experiments. The

Figure 1.

Fractionation by the modified method of Schmidt & Thannhauser (1945).

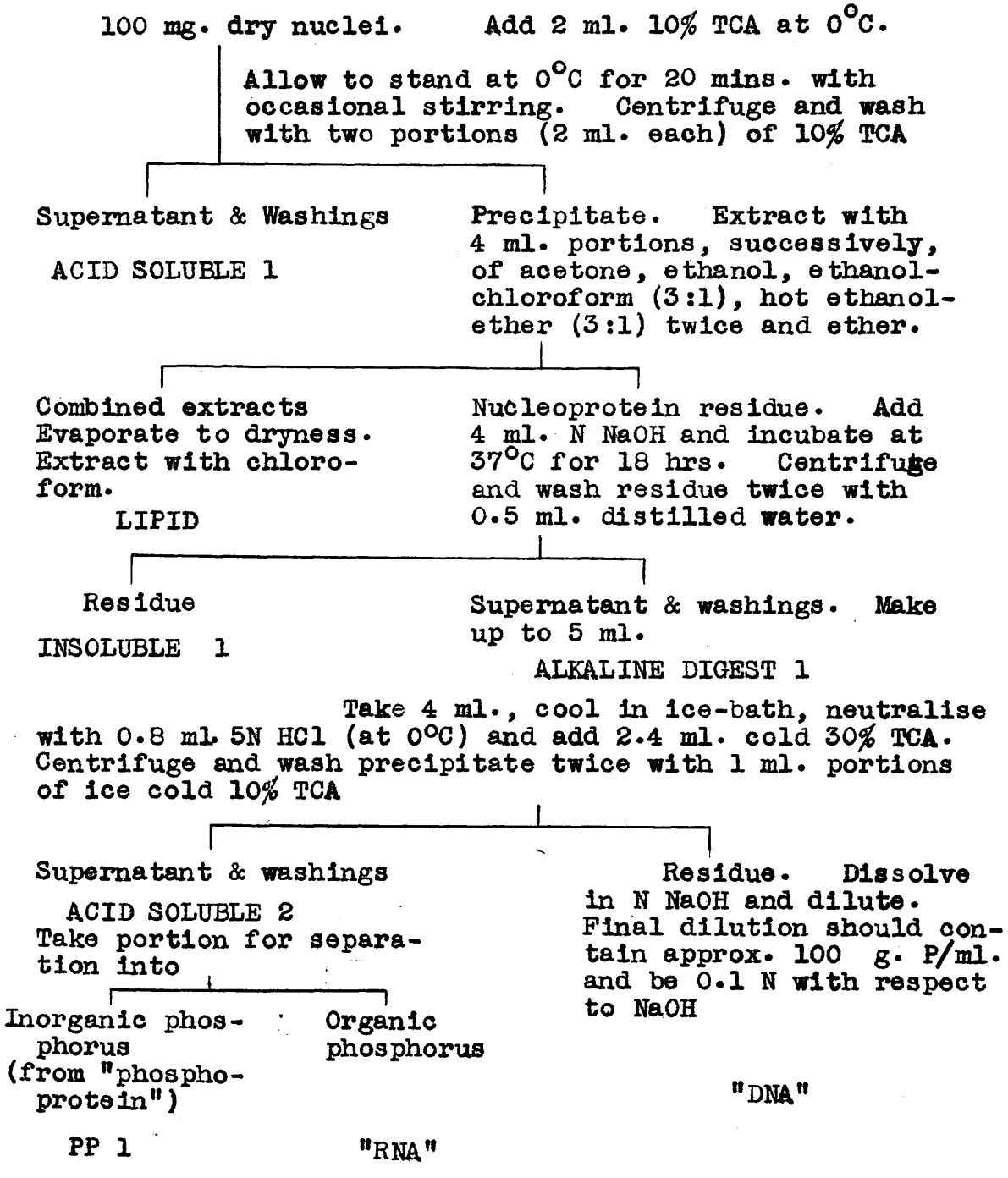


Figure 2.

Fractionation of the Schneider Residue.

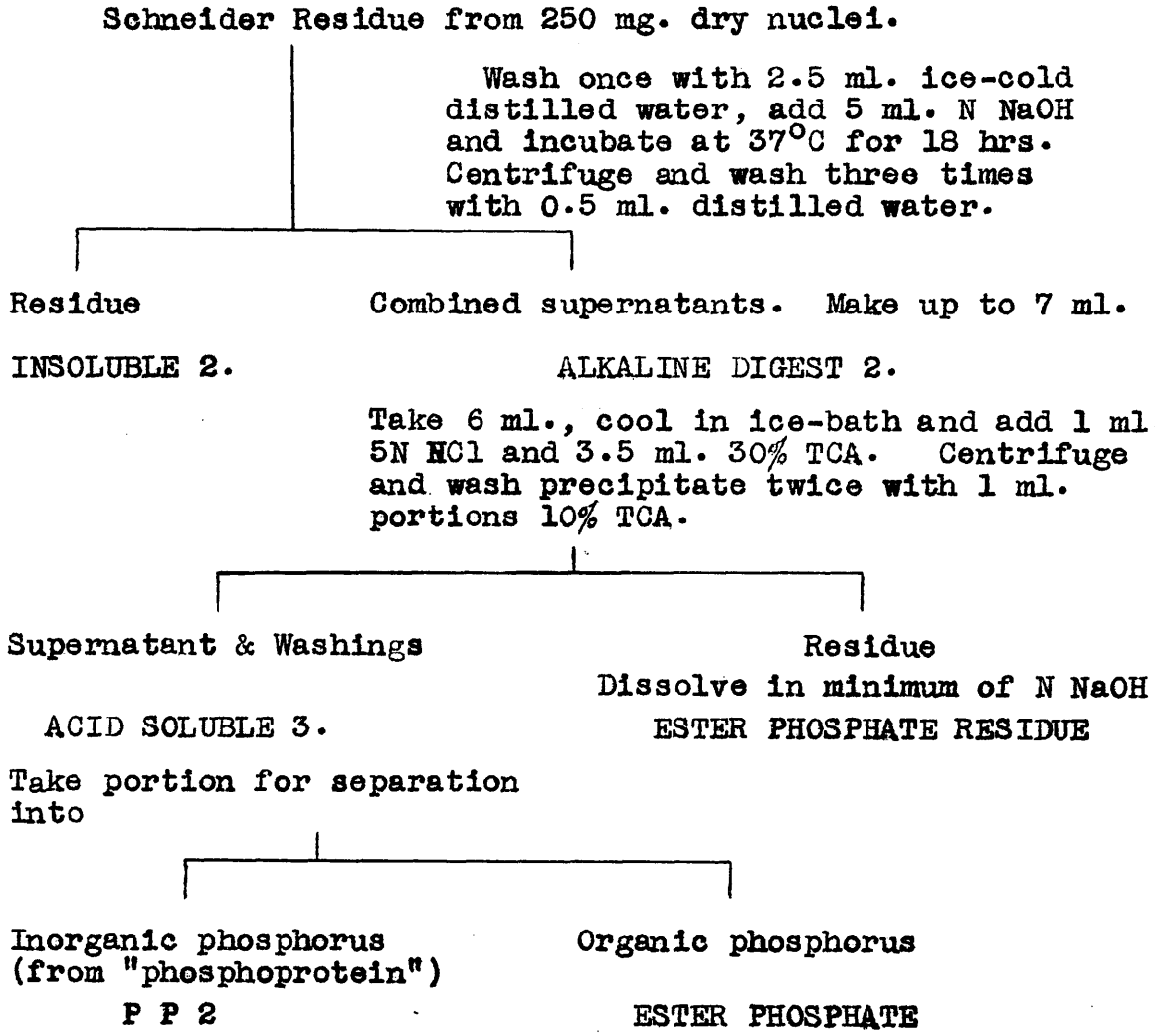


Table 19.

Analyses carried out on the various fractions of the Schmidt & Thannhauser, and Schneider procedures. *

Fractionation	Fraction	Presumed Constituents	Analyses
Schmidt & Thannhauser & Schneider	Acid Soluble 1	Inorganic phosphate & phosphate esters	Total phosphorus
	Lipid	including phospholipid	Total phosphorus.
Schmidt & Thannhauser	Insoluble 1		Total phosphorus
	Alkaline Digest 1	Inorganic phosphate, ribomononucleotides DNA	Total phosphorus
	Acid Soluble 2	Inorganic phosphate, ribomononucleotides	Total phosphorus. Inorganic phosphorus. Pentose by orcinol & phloroglucinol.
	"RNA"	Ribomononucleotides	Total phosphorus.
	"DNA"	DNA (& protein)	Total phosphorus DNA by diphenylamine & U.V.
Schneider	Schneider Extract	Hydrolysis products of RNA and DNA	Total phosphorus Pentose (orcinol & phloroglucinol) DNA (diphenylamine) U.V. absorption

Table 19 (Continued)

Fractionation	Fraction	Presumed Constituents	Analyses
Schneider	Schneider Residue	"Phosphoprotein"	Total phosphorus.
Schmidt & Thannhauser fractionation of Schneider Residue	Insoluble 2		Total phosphorus
	Alkaline Digest 2	Inorganic phosphate plus ester phosphate	Total phosphorus Diphenylamine test.
	Acid Soluble 3	Inorganic phosphate plus ester phosphate	Total phosphorus. Inorganic phosphorus. Pentose.
	Ester Phosphate	Unknown phosphorus compounds	Total phosphorus.
	Ester Phosphate residue	"	"

* In experiments in which radioactive phosphorus was used the radio activity of each fraction was determined. The determination of the radioactivity of the inorganic phosphate (PP1 and PP2) was made on the blue solution obtained in the estimation of phosphorus.

components of the RNA fraction obtained in the Schmidt and Thannhauser procedure were further separated by the method of ionophoresis on paper described by Smellie and Davidson (1951).

The nucleoprotein residue (100 mg.) was incubated in 0.5 ml. N KOH (in later experiments 1 ml. 0.3 N KOH was used) at 37°C for 18 hrs. The digest was cooled in an ice-bath and acidified with 60% perchloric acid. After 15 minutes the insoluble material ("DNA" fraction) was centrifuged out and washed once with 0.2 ml. ice-cold distilled water. The combined supernatants were brought to a pH of about 4.0 with N KOH. The precipitate of potassium perchlorate was centrifuged down and washed. The supernatant fluid contains mononucleatides derived from RNA and corresponds to the Acid Soluble 2 fraction previously described (Figure 1).

e. Treatment of the "DNA" fraction. The "DNA" fraction was washed twice with a little ice-cold 10% TCA to remove any acid soluble material which might remain together with excess perchloric acid. It was then dissolved in N NaOH and the solution diluted with distilled water. The potassium perchlorate which did not dissolve was centrifuged down, washed twice with a little distilled water and the combined supernatants cooled in an ice-bath. The solution was approximately neutralised with 5 N HCl and 0.5 vol. ice-cold

30% TCA added. The precipitate was centrifuged down, washed twice with a little ice-cold 10% TCA and dissolved in the minimum of N NaOH. The solution was then made up to 6 ml. and then used for the determination of radio-activity and phosphorus.

f. Ionophoresis of the Acid Soluble 2 fraction. With the aid of an 'Agla' micrometer syringe an accurately measured volume of solution, containing about 80 μg. total phosphorus, was applied in a narrow band (about 5 cm. long) across a strip of Whatman 3 MM. filter paper (72 cm. x 7 cm.) 5 cm. from one end. The paper was dried during the application by a current of warm air. The paper was then moistened with 0.02 M citric acid/trisodium citrate buffer at pH 3.5. (The section of paper containing the material applied was allowed to become moist by capillary attraction.) The strip of paper was then suspended over a glass rod so that the two ends dipped into buffer solution contained in two large glass dishes. Carbon electrodes connected to a direct current supply were placed in each dish so that the cathode was in the dish nearest the spot on the paper. The apparatus was enclosed in a glass case to minimise evaporation. To obtain adequate separation of the four nucleotides the run was carried out at a potential gradient of about 10.7 volts/cm. for 18 hours. On completion of the run the paper strip was dried in front of an infra-

red lamp or in an oven at 70°C. An example of the separation is given in Figure 3.

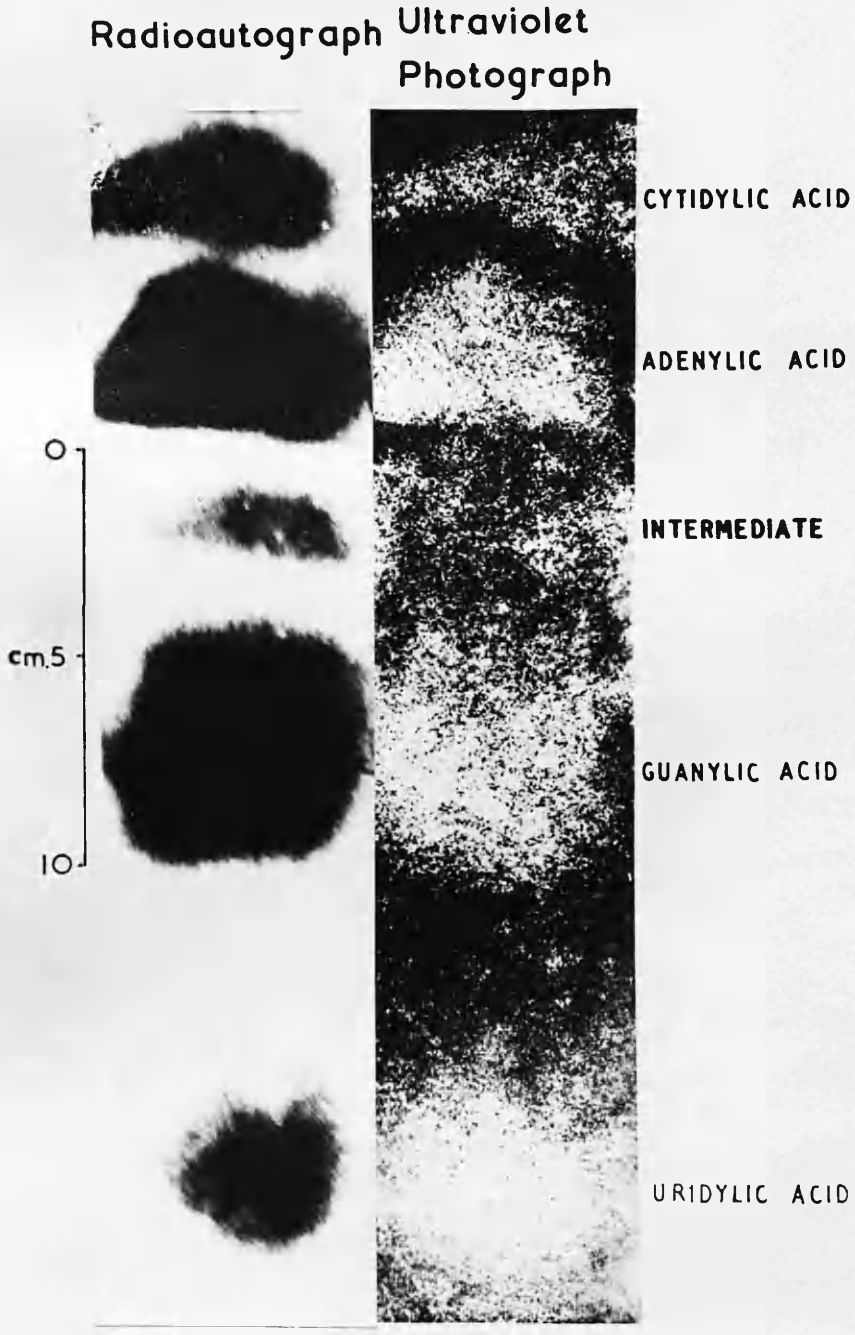
The ribonucleotides are preceded by components, including inorganic phosphate, which run off the end of the paper under the conditions described above. A separate run, at a potential gradient of 13.6 volts/cm. for six hours, was therefore necessary to obtain them. (Size of paper used: 57 cm. x 7 cm.). An example of this separation is given in Figure 4.

g. Location of Bands. The bands corresponding to each nucleotide and to the most rapidly moving component (Component 'X') were located on the paper with the aid of an ultraviolet lamp and special glass filter as described by Holiday & Johnson (1949) and were marked lightly in pencil.

h. Photographic method. A photographic record of most runs was made using the method of Markham & Smith (1949). The paper to be photographed was placed over a sheet of Ilford Reflex Contact Document 50 paper and pinned to a curved board to ensure good contact over its entire surface. It was exposed for 30 minutes to suitably filtered ultra-violet light from a low-pressure mercury vapour lamp at three feet distance. The document paper was developed, washed and dried in the usual way.

i. Radioautographs. In some cases radioautographs

Fig.3. Ionophoresis of Acid Soluble 2.
(long run)



of the ionophoresis runs were prepared using Kodak Industrex Type D film. The film was left in contact with the paper used in the ionophoresis run for eighteen days and developed and then printed. (See Figs. 3 and 4).

j. Elution of Material. The portions of paper containing the bands were cut out, trimmed to a point at one end and the material eluted into graduated tubes with 0.01 N NaOH (see Consden, Gordon & Martin, 1947). The nature of each eluate was determined by its position on the paper and its ultraviolet absorption spectrum. Total phosphorus and radioactivity were also determined.

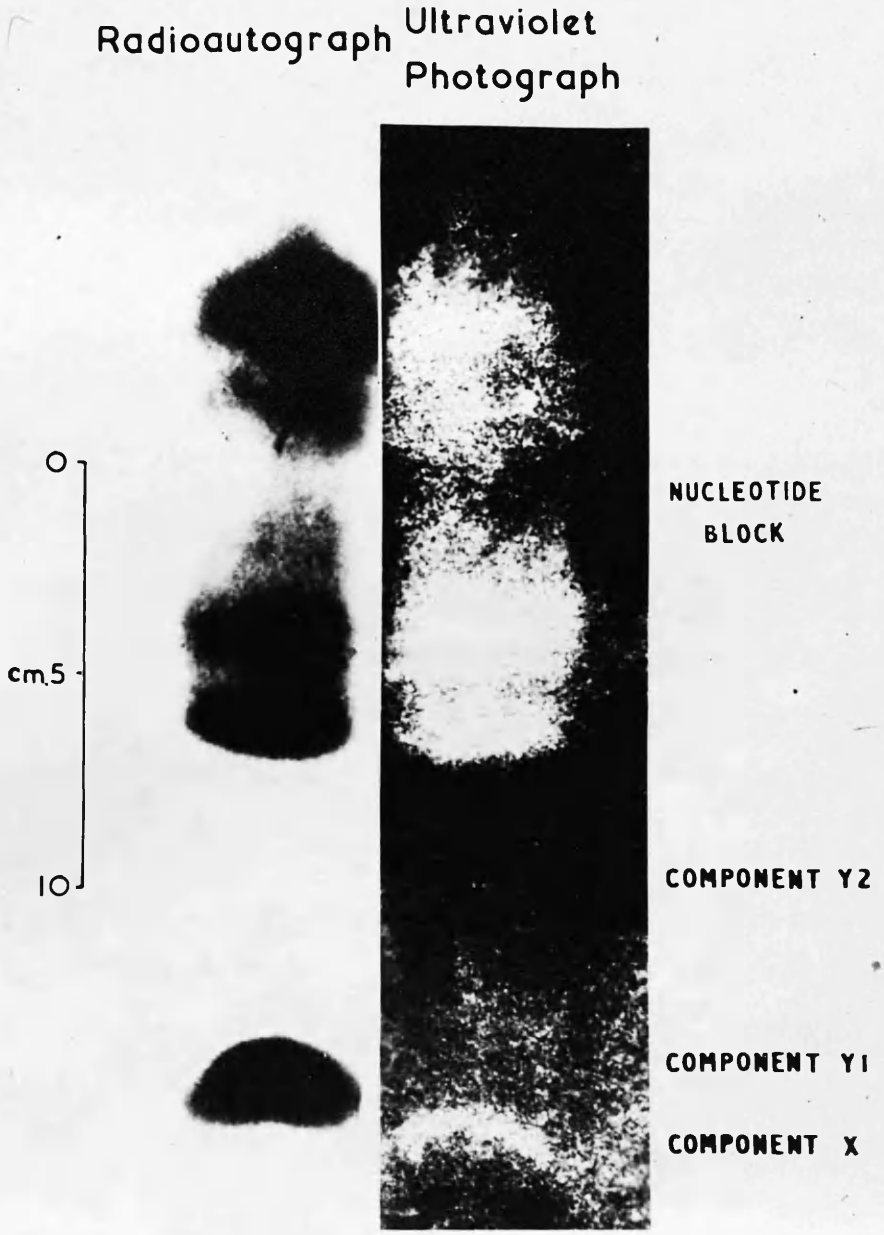
In experiments in which ^{32}P was employed the radioactivity of inorganic and organic phosphorus in "component X" was determined separately. Inorganic phosphorus was determined by adding acid, molybdate and amidol (see p. 40) to an undigested sample of the eluate and the blue colour measured in a Spekker photometer. The phosphomolybdate was then extracted with isobutanol and the radioactivity of the extract determined. The radioactivity of the aqueous layer was also measured and the amount of organic phosphate it contained obtained by difference, total phosphorus having been determined in another aliquot.

2.4. Preparation of Purified Nucleic Acids.

Purified nucleic acids were prepared for use as

Fig. 4. Ionophoresis of Acid Soluble 2.

(short run)



standards in the analytical determinations.

a. Preparation of Deoxyribonucleic Acid.
(Taylor, Greenstein & Hollaender, 1947).

500 g. calf thymus was suspended in ice cold distilled water in a cooled Waring blendor. The thymus was dealt with in three lots, 200 ml. water being used on each occasion, and the blendor run for 15 mins. each time. Unbroken lumps of tissue were resuspended in a little water for 20 mins. The homogenate (final volume 1200 ml.) was left over-night in the cold room. The suspension was filtered through cheese-cloth, one, two and then four layers being used. 1.5 ml. 20% $CaCl_2$ was added and the nucleoprotein precipitate allowed to settle in the cold room. The supernatant was siphoned off and the precipitate centrifuged at 1000 r.p.m. for 5 mins., and washed twice with ice-cold distilled water containing a little $CaCl_2$. 75 ml. 10% NaCl was added to the precipitate which was stirred until it was dissolved. The solution was saturated with sodium chloride (30 g.), 900 ml. saturated NaCl added and the mixture left overnight in the cold room. The precipitated protein was filtered off on a Buchner funnel with the aid of Celite and the filtrate, containing DNA, was allowed to run through a capillary into two volumes of 95% industrial spirit. The precipitate was centrifuged and washed with 70, 80, 90, 95 and 100% ethanol and finally ether. The preparation was

then air dried.

The DNA was freed from the last traces of RNA by incubation in N NaOH at 37°C for 18 hrs. The DNA was precipitated by acidification with HCl and addition of two volumes of ethanol. It was then centrifuged and washed with alcohol and ether and dried.

Phosphorus content 9.1%.

b. Preparation of Yeast Ribonucleic Acid.

A sample of commercial yeast nucleic (B.D.H.) was dissolved in the minimum of dilute alkali and purified by repeated precipitation with ten volumes of glacial acetic acid and finally washed with ethanol and ether and dried.

Phosphorus content 7.8%.

2.5. Analytical Methods.

a. Estimation of Phosphorus (modified method of Allan, 1940).

Reagents

(1) 10 N H_2SO_4

(2) 100 volume H_2O_2 (M.A.R.)

(3) Amidol reagent. 1 g. amidol was dissolved in 100 ml. of stock 20% sodium bisulphite and filtered. The amidol reagent was kept no longer than five days.

(4) 8.3% ammonium molybdate. To facilitate solution of the ammonium molybdate a little ammonia was added.

(5) Standard phosphate solution. A standard solution containing 1 mg. P/ml. was prepared by dissolving 1.0967 g. A.R. KH_2PO_4, dried in an air oven, in distilled water and made up to 250 ml. It was kept over chloroform at 0°C and was suitably diluted for the preparation of a calibration curve.

Procedure. The sample taken contained between 20 and 200 μg. phosphorus. It was heated in a micro-kjeldahl flask in a digestion rack with 1.2 ml. 10 N H_2SO_4. A few drops of 100 volume H_2O_2 were used to complete the digestion. The flask was cooled and 6.35 ml. distilled water added followed by 2 ml. amidol reagent, 1 ml. 8.3% ammonium molybdate and 15 ml. distilled water. The total volume was 25 ml. and the final concentration of sulphuric acid was 0.5 N. The colour was read between 10 and 30 mins. after addition of reagents in a Hilger spekker absortiometer using Ilford red filters (no. 608). For small amounts of phosphorus half or fifth quantities of reagents were used.

b. Estimation of Inorganic Phosphate in the Presence of Organic Phosphate.

Principle. The inorganic phosphate was precipitated as magnesium ammonium phosphate, the precipitate washed with ammonia and the phosphorus estimated by the Allen method.

Reagents.

Mathisons reagent (magnesium ammonium citrate) (Mathison 1909). 40 g. citric acid were dissolved in 500 ml. water and 20 g. light magnesium oxide added to the hot solution. After cooling 400 ml. 0.88 ammonia were added and the solution filtered after standing overnight.

Procedure. To each 10 ml. of solution used in the estimation 1 ml. of Mathison's reagent was added and the solution made alkaline to phenolphthalein with 0.88 ammonia and placed in the refrigerator overnight. The phosphate precipitate was centrifuged down and washed twice with a little 10% ammonia. Fifth quantities of reagents were used in the estimation of phosphorus.

c. The Estimation of DNA.

The diphenylamine reaction originally developed by Dische (1930) for the detection and estimation of DNA was used in the modification described by Davidson & Waymouth (1944).

Principle. The method depends on the release from DNA of a derivative of deoxyribose, probably ω -hydroxy-laevulenic aldehyde (Stacey, Deriaz, Teece & Wiggins, 1946). Only the deoxyribose of the purine nucleotides is reactive.

Reagents.

(1) 0.2 N HCl (A.R.)

(2) Diphenylamine reagent. 1 g. diphenylamine (A.R.) twice recrystallised from ethanol was dissolved in 2 ml. conc. H_2SO_4 (A.R.) and 98 ml. glacial acetic acid (A.R.) added. The reagent was kept in a dark bottle.

Method. The sample containing DNA or its derivatives (10 - 100 μ g. DNA phosphorus) was neutralised. When it was alkaline, as in the Schmidt & Thannhauser method, 0.2 N HCl was used; when it was acid, as in the Schneider procedure, N NaOH was employed. The sample, made up to a volume of 1 ml. was treated with 1 ml. 0.2 N HCl and heated in a boiling water-bath for 15 mins. After cooling, 4 ml. diphenylamine reagent were added, the solution mixed thoroughly and heated in the water-bath for 6 mins. The colour intensity was estimated using a Hilger spekker absorptiometer and Ilford yellow filters (no. 606). A blank containg distilled water in place of the DNA solution was run with each batch.

Serial dilutions of a solution of purified calf thymus DNA (1 mg./ml.) were used for the preparation of a calibration curve. Absorptiometer readings were plotted against DNA phosphorus.

d. The Estimation of RNA.

Two methods for the estimation of RNA based on reactions for pentose were used. One was the orcinol method as used by Kerr & Seraidarian (1945) and the other the phloroglucinol method of Euler & Hahn (1946). The latter has the advantage that DNA does not interfere.

Principle. Purine-bound pentose liberated by acid hydrolysis of RNA is converted to furfural which reacts with a phenol to give a characteristic colour.

Orcinol method.

Reagents.

(1) Stock ferric chloride reagent. 0.02% $FeCl_3.6H_2O$ in conc. HCl.

(2) Orcinol reagent. 60 mg. orcinol dissolved in 10 ml. ferric chloride reagent. This reagent was made up daily.

Procedure. To 3 ml. test solution containing 1-8 μg. RNA phosphorus were added 3 ml. orcinol reagent. The solution, after thorough mixing, was heated in a boiling water-bath for 30 mins. After cooling the colour was read off in a Hilger spekker absorptiometer using Ilford red filters (no. 608). A blank, using water instead of the pentose-containing solution, was run with each batch.

A calibration curve was prepared from serial dilutions of a solution of RNA containing 0.1 mg. RNA/ml. The colour intensities produced were plotted against the corresponding amount of RNA phosphorus over the range

1-8 μg. P.

Phloroglucinol Method.

Reagents.

(1) Stock Ferric Chloride Solution. 0.1% $FeCl_3.6H_2O$ in a mixture of 1 part HCl and 6 parts glacial acetic acid.

(2) Phloroglucinol Reagent. 0.25% phloroglucinol in a mixture of 1 part conc. HCl, 1 part distilled water and 2 parts glacial acetic acid. The reagent was kept in a dark bottle.

Procedure. 1 ml. samples containing less than 100 μg. RNA phosphorus were transferred to boiling tubes (6" x 1"). To each sample and a blank (1 ml. distilled water) 8 ml. of ferric chloride solution was added. After thorough mixing the tubes were placed in a boiling water-bath for 50 mins. and then cooled. 1 ml. of phloroglucinol reagent was added, the solution mixed immediately and kept at room temperature for exactly 20 mins. The tubes were now immersed in a boiling water-bath for 4 mins., cooled and kept at room temperature for 2-24 hrs. The colour intensity was read in a Hilger spekker absorptiometer using Ilford red filters (no. 608).

The calibration curve was obtained in the same way as for the Orcinol method except that the yeast RNA solution contained 2 mg. RNA/ml. The range covered was 10-100 μg. RNA phosphorus.

In preparing calibration curves for the orcinol and phloroglucinol methods it was found that RNA which had been incubated in alkali gave a slightly higher absorption per unit of phosphorus than that which had not. Two calibration curves were therefore prepared for each method. One was prepared using standard yeast RNA and the other from standard yeast RNA which had been incubated in N NaOH at 37° for 18 hrs. and treated with TCA as in the Schmidt & Thannhauser procedure. TCA does not interfere in either the orcinol or phloroglucinol method.

e. Determination of the Ultraviolet Absorption of Fractions Containing RNA and DNA.

Ultraviolet absorption was determined using a Beckman DU spectrophotometer or a Unicam SP 500 quartz spectrophotometer at wavelengths of 2600 A and 2900 A. The difference in the readings obtained was proportional to the amount of nucleic acid present. The sample to be examined was diluted so that it contained less than 4 μg. nucleic acid phosphorus per ml. and read against a reagent blank. (TCA, which absorbs ultraviolet light, was usually present in the test solution and was compensated for in the blank).

To obtain calibration curves samples of purified RNA and DNA were submitted to the Schmidt & Thannhauser procedure, suitable dilutions of the nucleic acid made and each read

against a reagent blank diluted similarly.

The Schneider Extract contains bases derived from both RNA and DNA and the absorption of these solutions was computed from the calibration curves for RNA and DNA using the results from the colourimetric reactions for pentose and deoxypentose as an estimate of the relative amounts of the two nucleic acids present. The nature of the bases in the Acid Soluble 3 fraction is unknown and the absorption of this material was read off on the DNA calibration curve.

These methods for translating the ultraviolet absorption of the Schneider Extract and Acid Soluble 3 fraction into phosphorus values are not entirely satisfactory and the results should be considered as approximate.

2.6. Isotope Techniques.

Radioactive phosphorus was obtained from Harwell in the form of carrier free phosphoric acid solution. This was diluted to a concentration of about 200 μc./ml. before use.

With rabbits and rats the ^{32}P solution was injected subcutaneously between the shoulder blades. Fowls were given intramuscular injections. In general the dose administered was 10 μc./100 g. body weight but for experiments involving ionophoresis the dose was doubled or trebled. In all cases the animals were killed two hours after injection.

Determination of Radioactivity.

For the determination of radioactivity a known volume

of each solution was pipetted into a liquid counter (Type M 6 manufactured by Messrs. 20th Century Electronics) connected to a probe unit and scaling unit (Type 200 manufactured by Messrs. Dynatron Radio Ltd.). Radioactivity was recorded for ten minutes and all specific activities were calculated as counts/min./100 μg.P.

Section 3. Results.

3.1. Composition of Cell Nuclei. Results expressed in terms of dry weight.

The results of a comprehensive series of analyses are expressed in Tables 20 to 27 in terms of μg. P per 100 mg. dry weight of nuclei. All analyses were carried out in duplicate except those on Insoluble 1 and P.P.1.

The amount of acid soluble P (Acid Soluble 1) is in all cases small and the results probably have no significance since most of the acid soluble P would be lost during the isolation of the nuclei.

Before the composition of individual types of nuclei is considered it can be stated that the presence of RNA in the nucleus has been clearly established. This will be apparent from estimations of phosphorus, pentose and ultraviolet absorption and more particularly from the results of the ionophoresis experiments.

The data obtained for fowl erythrocyte nuclei are given in Tables 20 and 21. Since there seems to be no obvious difference between those of normal and tumour-bearing birds, Tables 20 and 21 will be considered together.

Practically all the phosphorus of the fowl erythrocyte nucleus is present as DNA (about 90%) and almost the whole of the remainder is divided between RNA and phospholipid.

Table 20.

Analyses of Erythrocyte Nuclei from Normal Fowls.
Results expressed as μg.P/100 mg. dry weight.

Fraction	Preparation							No. of Analyses
	FE 2	FE 3	FE 6	FE 8	FE 9	FE10	Mean	
Acid Soluble 1	43.7	26.7	23.3	16.1	19.1	10.4	23.4	6
Phospholipid	206	101	144	175	195	185	168	6
Insoluble 1	2.7	1.6		0.8	1.0	1.2	1.5	5
Alkaline Digest 1	2470	2440*		2770	2760*	2730	2630	5
Acid Soluble 2 Total P	182	203		267	448	96.5	239	5
RNAP by Orcinol		88.1		275	180	105	162	4
" " Phloroglucinol		90.0			170	118	126	3
" " U.V.Absorption						87.2		
",Organic P	175	191		263	409	81.6	224	5
P.P.1 (Inorganic P)	7.1	5.0		2.6	14.3	3.7	6.6	5
DNAP by Phosphorus	2230	2240		2540	2320	2730	2410	5
" " Diphenylamine		2380		2560	1990	2390	2330	4
" " U.V.Absorption						2580		
Schneider Extract. Total P		2070	1870	2030	2020	2410	2120	5
RNAP by Phloroglucinol		100		221	112	149	146	4
DNAP " Diphenylamine		2440		2320	1750	2420	2230	4
NAP " U.V. Absorption						2120		
Schneider Residue		283	199	614	528	359	397	5

* By calculation.

Table 21.

Analysis of Erythrocyte Nuclei from Fowls bearing the GRCH 15 Tumour.
Results expressed as μg.P/100 mg. dry weight.

Fraction.	Preparation					No. of Analyses.
	FE 1	FE 4	FE 5	FE 7	Mean	
Acid Soluble 1	84.0	16.3	7.3	28.5	34.0	4
Phospholipid	176	158	89	160	146	4
Insoluble 1	0.8	1.0	1.7	1.8	1.3	4
Alkaline Digest 1	2430	3090	2720*	2730	2740	4
Acid Soluble 2.						
Total P	94	80	182*	172	132	4
RNAP by Orcinol		95	123	165	128	3
" " Phloroglucinol		123	161	149	144	3
", Organic P	80	68	176	161	121	4
P.P.1 (Inorganic P)	0.8	11.6	5.5	8.3	6.6	4
DNAP by Phosphorus	2390	3020	2540	2550	2625	4
" " Diphenylamine		2370	2560	2315	2415	3
Schneider Extract.						
Total P		2620	2390	2150	2390	3
RNAP by Phloroglucinol		94	135	146	125	3
DNAP " Diphenylamine		2430	3080	2520	2680	3
Schneider Residue		356	358	461	392	3

* By calculation.

The amount of phosphorus present in the RNA fraction (RNAP, organic P) is often higher than would be expected on the basis of the pentose estimations and there is no constant relationship between the two sets of figures. This suggests either (1) the presence of a variable quantity of an unknown phosphorus compound and/or (2) the loss of a small amount of DNA into the Acid Soluble 2 fraction. (The appearance of 2% of the DNA in this fraction would increase its phosphorus content by about 40%). It should also be noted that the colour intensity of the final solutions obtained in the estimation of pentose by the phloroglucinol reaction was very low and hence the results based on this reaction are approximate.

A small amount of "phosphoprotein" phosphorus (P.P.1) is present in the Acid Soluble 2 fraction as inorganic phosphate. This does not account for the discrepancy between the total phosphorus of this fraction and RNAP estimates based on pentose determinations.

Generally the amount of DNA as estimated by the diphenylamine reaction confirms direct phosphorus analysis on the DNA fraction. The possibility of the presence of relatively small amounts of other phosphorus compounds in this fraction is however not excluded.

The phosphorus content of the Schneider Extract is much lower than the sum of the organic phosphorus of Acid

Soluble 2 plus DNA phosphorus. On the other hand the Schneider Residue shows a much higher phosphorus content than would be accounted for by "phosphoprotein" (i.e., P.P.1) alone.

The composition of calf thymus nuclei (Table 22) is similar to that of fowl erythrocyte nuclei, but the phospholipid content is much lower and P.P.1 shows a value about twice that for the erythrocyte nuclei. (Results obtained by the phloroglucinol reaction are again approximate due to the small amount of RNA present in Acid Soluble 2).

The Acid Soluble 2 fraction contains inorganic phosphate (P.P.1) derived from "phosphoprotein". It is also evident that the organic phosphorus of this fraction is much greater (about 50%) than RNAP obtained by pentose estimations. (Results obtained by the phloroglucinol reaction are again approximate due to the low concentration of RNA). This could be due to the presence of unknown phosphorus compounds, but it could also be explained if the RNA had a much lower purine to pyrimidine ratio than the yeast RNA used as a standard. In preparation CT 4b the discrepancy between RNA phosphorus by the two methods is very large and DNA (or its derivatives) was detected in the Acid Soluble 2 fraction and estimated by the diphenylamine reaction. If the assumption is made that the DNA or its derivatives present in the Acid Soluble 2 fraction has the same composi-

Table 22.

Analysis of Calf Thymus Nuclei
Results expressed as μg.P/100 mg. dry weight.

Fraction	Preparation							No. of Analyses
	CT 1	CT 2	CT 3	CT4a	CT 4b	CT 4c	Mean	
Acid Soluble 1	34.7	33.2	13.0	11.8	14.5	14.3	20.3	6
Phospholipid	7.9	19.4	23.3	34.4	17.9	30.0	22.2	6
Insoluble 1	1.4		1.3	0.7	0.2	1.2	1.0	5
Alkaline Digest 1	2420*	2080	2790	2820	2930	2810	2630	6
Acid Soluble 2.								
Total P	307*	263	356	169	494+	195	297	6
RNAP by Orcinol	207		191	124	115**	131	154	5
" " Phloroglucinol	196		213	103	131	108	150	5
" " U.V.Absorption					489	177		
",Organic P	297		353	173	439	161	285	5
P.P.1.	9.9		10.1	3.3	15.6	23.0	12.4	5
DNAP by Phosphorus	2110	1725	2470	2660	2520	2610	2350	6
" " Diphenylamine	2100		2810	2370	2290	2480	2410	5
" " U.V. Absorption					2530	2550		
Schneider Extract.								
Total P	2060	1890		2170	2360	2320	2160	5
RNAP by Orcinol	126			122	106	111	116	4
" " Phloroglucinol	173			84	111	101	117	4
DNAP " Diphenylamine	2420			2280	2340	2460	2380	4
NAP " U.V.Absorption					2150	2010		
Schneider Residue	262	226		624	523	447	414	5

* By calculation.

** Corrected for DNA

+ DNAP by the diphenylamine reaction 391

tion as the bulk of the DNA (and this is not necessarily so) then, in this case, DNA could account for all or nearly all of the phosphorus in Acid Soluble 2 not accounted for as inorganic phosphate and RNAP by pentose reactions. Estimates based on ultraviolet absorption measurements indicate that practically all of the phosphorus in Acid Soluble 2 is in the form of nucleotides. This seems to be true of the DNA fraction also.

As for the fowl erythrocyte nuclei, the Schneider Extract of calf thymus nuclei contains less phosphorus and the Schneider Residue more phosphorus than would be expected.

The general picture presented by analysis of rabbit liver nuclei (Table 23) is similar to those given previously. However, the amount of DNA present is very much lower and the content of RNA is about 60% greater. P.P.1 and RNAP by pentose reactions does not apparently account for the phosphorus of Acid Soluble 2 in all cases. The discrepancy, however, is not nearly as great as in calf thymus nuclei.

The higher content of RNA of rabbit liver nuclei is more pronounced in rat liver nuclei (Table 24) so that of the total nucleic acid about 20% is RNA. P.P.1 shows an even greater increase. In rats fasted for 72 hours (Table 25) the RNA content lies at the lower end of the range for fed animals. This suggests a loss of RNA on fasting which will be more apparent in Tables 40 and 41 in which the results are expressed per nucleus.

Table 23.

Analysis of Liver Nuclei from Normal Rabbits.
Results expressed as μg.P/100 mg. dry weight.

Fraction	Preparation RL 1	RL 2	RL 3+	RL 4	RL 5	RL 6	Mean	No. of Analyses
Acid Soluble 1	18.2	17.3	25.1	15.1	26.7	17.4	20.0	6
Phospholipid	19.1	59.2	99.0	123	103	60.3	77.3	6
Insoluble 1	1.0	6.5	8.1	1.1	0.0	1.6	3.1	6
Alkaline Digest 1	2090*	1890	1750	1530	1790	1640	1780	6
Acid Soluble 2. Total P	269	310	508	316	293	249	324	6
RNAP by Orcinol	264	224		250	261	200	240	5
" " Phloroglucinol	235	216		271	263	241	245	5
" " U.V.Absorption					241	215		
" , Organic P	259	275	436	280	255	212	286	6
P.P.1 (Inorganic P)	16.1	21.2	7.7	12.4	10.0	22.6	15.0	6
DNAP by Phosphorus	1820	1610	1350	1170	1570	1370	1480	6
" " Diphenylamine	1890	1670		1210	1690	1250	1540	5
" " U.V. Absorption					1750	1290		
Schneider Extract. Total P	1710	1430		1260	1390		1450	4
RNAP by Orcinol	181	193		212	211		199	4
" " Phloroglucinol	237	202		199	202		220	4
DNAP " Diphenylamine	2050	1540		1350	1650		1650	4
NAP " U.V.Absorption					1290			
Schneider Residue	316	405		220	364		326	4

* By calculation. + Amount of material analysed was small.

Table 24.

Analysis of Liver Nuclei from Normal Male Rats on Stock Diet.
Results expressed as μg.P/100 mg. dry weight.

Fraction	Preparation 41	55	58	63	64	Mean	No. of Analyses.
Acid Soluble 1	24.5	22.7	25.0	19.2	24.0	23.1	5
Phospholipid	164	62.8	117	48.9	112	101	5
Insoluble 1	1.0	1.2	0.9	2.4	1.4	1.4	5
Alkaline Digest 1	1460	1750	1510	1770	1640	1630	5
Acid Soluble 2.							
Total P	392	339	394	327	439	378	5
RNAP by Orcinol	372	309	313	263	390	329	5
" " Phloroglucinol		328	329	265	346	317	4
" " U.V.Absorption		309	343	276	357	321	4
" , Organic P	371	288	358	297	390	341	5
P.P.1 (Inorganic P)	31.0	40.3	22.6	13.6	24.5	26.4	5
DNAP by Phosphorus	1000	1440	1160	1450	1240	1260	5
" " Diphenylamine	1160	1240	988	1320	1140	1170	5
" " U.V. Absorption		1420	1190	1530	1220	1340	5
Schneider Extract.							
Total P	1120	1390	1240	1540	1340	1330	5
RNAP by Orcinol	339	275	304	238	368	305	5
" " Phloroglucinol	354	248	246	234	284	273	5
DNAP " Diphenylamine	1170	1100	726	1440	987	1080	5
NAP " U.V.Absorption		1340	1310	1440	1220	1330	4
Schneider Residue	273	312	258	288	272	281	5

Table 25.

Analysis of Liver Nuclei from Normal Male Rats Fasted for 72 hours.
Results expressed as μg.P/100 mg. dry weight.

Fraction.	Preparation 43	52+	57	61	Mean	No. of Analyses.
Acid Soluble 1	16.9	13.7	21.3	18.5	17.6	4
Phospholipid	39.8	196	54.9	66.8	89.4	4
Insoluble 1	2.3	1.0	1.7	0.9	1.5	4
Alkaline Digest 1	1790	1440	1830	1710	1690	4
Acid Soluble 2. Total P	325*	410	318	315	342	4
RNAP by Orcinol	236	359	263	266	281	4
" " Phloroglucinol	309	365	264	242	295	4
" " U.V.Absorption		390	245	274	303	3
", Organic P	294	380	308	265	312	4
P.P.1 (Inorganic P)	30.7	14.1	7.5	38.7	22.8	4
DNAP by Phosphorus	1340	1040	1480	1410	1320	4
" " Diphenylamine	1300	917	1550	1310	1270	4
" " U.V. Absorption		1090	1440	1380	1300	3
Schneider Extract. Total P	1330	1120	1560	1400	1350	4
RNAP by Orcinol	223	321	254	249	262	4
" " Phloroglucinol	272	306	237	228	261	4
DNAP " Diphenylamine	1330	820	1410	1370	1230	4
NAP " U.V. Absorption		1030	1430	1370	1280	3
Schneider Residue	348	258	334	306	312	4

* By calculation + Fasted for 48 hours.

The composition of liver nuclei (one sample, No.59) from female albino rats shown in Table 26 does not indicate any significant difference from that of the liver nuclei of male rats. The same applies to the specimen of liver nuclei from hooded male rats (No.70). The nuclei of regenerating liver (male albino rats: preparation 45) show no obvious differences in composition from those of normal rat liver. (But see experiments involving ^{32}P, Section 3.4).

The phosphorus of the Acid Soluble 2 fraction of rat liver nuclei seems to consist, in most cases, almost entirely of P.P.1 and RNAP (as determined by pentose estimation). However, in some preparations (63, 57 and 45), phosphorus which is not accounted for by these components appears to be present.

The GRCH 15 fowl tumour is an example of a very rapidly growing tissue and the analysis of one sample of nuclei obtained from it is shown in Table 27 (see also Table 43) together with the analysis of nuclei prepared from the liver of the same bird. The composition of the liver nuclei is similar to that of rabbit and rat liver nuclei, but the tumour nuclei are markedly different from any others analysed. The DNA content is extremely low and hence the phosphorus content in terms of phospholipid and RNA is of the same order as that for DNA. The RNA content as estimated by pentose determination is considerably higher than that indicated by

Table 26.

Analysis of Rat Liver Nuclei.

Results expressed as μg.P/100 mg. dry weight.

Fraction	Preparation		
	59. Female Albino	70. Male Hooded	45. Male Albino Regenerating Liver
Acid Soluble 1	22.0	22.5	11.5
Phospholipid	124	47.7	42.9
Insoluble 1	1.1	1.7	1.7
Alkaline Digest 1	1580	1800	1650
Acid Soluble 2.			
Total P.	403	400	434*
RNAP by Orcinol	341		328
" " Phloro-glucinol	334		293
" " U.V. Absorption	325		
" , Organic P	357	360	391
P.P.1 (Inorganic P)	38.1	22.4	43.3
DNAP by Phosphorus	1260	1300	1150
" " Diphenylamine	1120		998
" " U.V. Absorption	1250		
Schneider Extract.			
Total P.	1350		1310
RNAP by Orcinol	325		227
" " Phloroglucinol	281		233
DNAP " Diphenylamine	860		1280
NAP " U.V. Absorption	1210		
Schneider Residue	261		315

* By calculation.

Table 27.

Analysis of the Nuclei of Tumour and Liver of a Fowl bearing GRCH 15 Tumour.
Results expressed as μg.P/100 mg. dry weight.

Fraction	Preparation	
	Liver	Tumour
Acid Soluble 1		24.1
Phospholipid	46.2	190
Insoluble 1	2.1	1.5
Alkaline Digest 1	2050	550
Acid Soluble 2. Total P.	364	291
RNAP by Orcinol	285	340
" " Phloroglucinol	273	330
" " U.V. Absorption	297	246
" , Organic P	317	281
P.P.1 (Inorganic P)	35.4	5.1
DNAP by Phosphorus	1780	236
" " Diphenylamine	1620	200
" " U.V. Absorption	1680	264

organic phosphate and ultraviolet absorption determinations.

Although the tumour nuclei are difficult to prepare and although the preparation was not as clean as those obtained from other tissues, it is unlikely that the degree of contamination was great enough to be entirely responsible for the unusual pattern observed.

Throughout the present series of analyses of nuclei a small insoluble residue (Insoluble 1) was observed after alkaline digestion of the nucleoprotein. The amount of phosphorus present is very small compared with that in any other fraction. A similar residue has been observed in work with whole tissue. When phospholipid is extracted with chloroform from the dried total lipid fraction, an insoluble residue remains which, in rat liver nuclei, contains protein or protein breakdown products. This material is extracted from the acid washed nuclei in the first lipid extraction (i.e., the acetone extraction). It contains a very small amount of phosphorus which may or may not represent phospholipid contamination.

In several cases the pentose reactions carried out on the Schneider Extract give lower values for RNA than those done on Acid Soluble 2. This may be due to pretreatment with hot TCA but is more likely due to failure of the Schneider extraction procedure to remove all the nucleic

acid derivatives from the protein residue. Estimates of DNA by the diphenylamine reaction on the "DNA" fraction and on the Schneider Extract agree fairly well but the diphenylamine reaction sometimes gives slightly lower values for DNA than those obtained by direct phosphorus analysis in the Schmidt & Thannhauser procedure. However the discrepancy is not great enough to indicate the presence of phosphorus compounds in the "DNA" fraction other than DNA. Estimates of DNA based on ultraviolet absorption measurements agree with those from phosphorus determinations.

A summary of the results presented in Tables 20 to 25 is given in Table 28. Fowl erythrocyte and calf thymus nuclei are distinguished from liver nuclei by their higher DNA content and lower RNA content. P.P.1 follows the RNA content being greater when RNA is greater.

The Schneider Extract always contains less phosphorus and ultraviolet absorbing material than can be accounted for by RNA and DNA. On the other hand the Schneider Residue always contains much more phosphorus than is accounted for as "phosphoprotein" phosphorus (P.P.1). This would suggest that hot TCA does not extract all the nucleic acid phosphorus from the nucleoprotein residue and an investigation of this possibility is discussed in the next section.

Table 28.

Analysis of Nuclei : Summary.

Results expressed as μg.P/100 mg.

Fraction	Type of Nuclei: Fowl (Normal) Erythrocytes	Fowl (Tumour-bearing) Erythrocytes	Calf Thymus	Rabbit Liver	Rat (Fed) Liver	Rat (Fasted) Liver
Acid Soluble 1	23.4	34.0	20.3	20.0	23.1	17.6
Phospholipid	168	146	22.2	77.3	101	89.4
Insoluble 1	1.5	1.3	1.0	3.1	1.4	1.5
Alkaline Digest 1	2630	2740	2630	1780	1630	1690
Acid Soluble 2. Total P	239	132	297	324	378	342
RNAP by Orcinol	162	128	154	240	329	281
" " Phloroglucinol	126	144	150	245	317	295
" " U.V. Absorption					321	303
", Organic P	224	121	285	286	341	312
P.P.1 (Inorganic P)	6.6	6.6	12.4	15.0	26.4	22.8
DNAP by Phosphorus	2410	2625	2350	1480	1260	1320
" " Diphenylamine	2330	2415	2410	1540	1170	1270
" " U.V. Absorption					1340	1300
Schneider Extract. Total P	2120	2390	2160	1450	1330	1350
RNAP by Orcinol			116	199	305	262
" " Phloroglucinol	146	125	117	220	273	261
DNAP " Diphenylamine	2230	2680	2380	1650	1080	1230
NAP " U.V. Absorption					1330	1280
Schneider Residue	397	392	414	326	281	312

3.2. Analysis of the Schneider Residue.

The high phosphorus content of the Schneider Residue and the expectation that the "phosphoprotein" phosphorus (P.P.1) present in Acid Soluble 2 would exist in a bound form in the Schneider Residue led to an investigation of this fraction using the Schmidt & Thannhauser procedure.

The results (Tables 29 to 34) are similar for each type of nucleus and they will be considered together. They are summarised in Table 35.

A small insoluble residue (Insoluble 2) is again apparent after digestion in alkali and the amount of phosphorus it contains is of the same order as that in Insoluble 1.

The greater part (75-90%) of the phosphorus of the Schneider Residue is acid soluble after incubation in alkali. Acid Soluble 3 contains a small amount of inorganic phosphate (P.P.2) which probably corresponds to P.P.1 obtained in the Schmidt & Thannhauser procedure although it is slightly more abundant. In liver nuclei from rats on stock diet and from fasted rats P.P.2 is greater than P.P.1 by about 30% and 50% respectively. There is apparently no great difference in rabbit liver nuclei.

Acid Soluble 3 was tested for pentose by the orcinol and phloroglucinol reactions. The colour obtained in the phloroglucinol reaction was of very low intensity and on many occasions was obviously influenced by interfering

factors. The orcinol reaction gave more satisfactory absorptiometer readings but again there appeared to be interference by substances other than pentose. It would be true to say that the orcinol reaction was always positive whereas the phloroglucinol reaction, usually doubtful, was sometimes negative. The impression gained is that if reactive pentose is present it is present in very small and variable amounts and that the greater values indicated by the orcinol reaction were perhaps due to pyrimidine-bound deoxypentose. Tests for deoxypentose (bound to purine) by the diphenylamine reaction carried out on Alkaline Digest 2 were in every case negative. The Acid Soluble 3 fraction does, however, show appreciable absorption of ultraviolet light.

Of the total phosphorus of the Schneider Residue the amount still remaining in an acid insoluble form (Ester Phosphate Residue) is of the order of 10 to 25%. The absorption spectrum of the fraction in ultraviolet light indicates the absence of purines and pyrimidines.

It is evident that the Schneider extraction procedure does not remove all the RNA and DNA from nucleoprotein leaving only phosphoprotein phosphorus behind as Schneider himself believed.

Table 29.

Analysis of Schneider Residue : Fowl Erythrocyte Nuclei.

Results expressed as µg.P/100 mg. dry weight of nuclei.

	Preparation					
	FE 6	FE 8	FE 9	FE 10	FE 7*	Mean
Fraction.						
Insoluble 2	1.0	0.7	1.0	4.5	0.6	1.6
Alkaline Digest 2	198	613	527	355	460	431
Acid Soluble 3. Total P	178	537	501	328	415	392
Ester Phosphate	171+	531	450	299	379	366
NAP by U.V. Absorption				128		
P.P.2 (Inorganic P)	7.6	14.2	19.2	14.0	21.4	15.5
Ester Phosphate Residue	24.0	62.3	49.5	31.9	49.8	43.5

* Tumour-bearing bird.

\+ By calculation.

Table 30.

Analysis of Schneider Residue : Calf Thymus Nuclei.

Results expressed as μg.P/100 mg. dry weight of nuclei.

	Preparation				
	CT 2	CT 4a	CT 4b	CT 4c	Mean
Fraction					
Insoluble 2		1.6	0.5	1.2	1.1
Alkaline Digest 2	226	622	522	446	454
Acid Soluble 3. Total P	193	570	457	418	410
Ester Phosphate	181*	504	408	366	365
NAP by U.V. Absorption			163	136	
P.P.2 (Inorganic P)	11.7	23.9	18.5	37.1	22.8
Ester Phosphate Residue	30.5	55.9	63.7	48.2	49.6

* By calculation.

Table 31.

Analysis of Schneider Residue : Rabbit Liver Nuclei.

Results expressed as μg.P/100 mg. dry weight of nuclei.

	Preparation			
	RL 2	RL 4	RL 5	Mean
Fraction				
Insoluble 2	1.7	0.9	0.5	1.0
Alkaline Digest 2	403	219	364	329
Acid Soluble 3. Total P	333	186	334	284
Ester Phosphate	289	141	272	234
NAP by U.V. Absorption			118	
P.P.2 (Inorganic P)	18.6	19.0	13.0	16.9
Ester Phosphate Residue	65.8	22.0	48.9	45.6

Table 32.

Analysis of Schneider Residue : Liver Nuclei from Normal Male Rats on Stock Diet.

Results expressed as µg.P/100 mg. dry weight of nuclei.

	Preparation					
	41	55	58	63	64	Mean
Fraction						
Insoluble 2	2.3	0.9	0.8	1.8	1.0	1.4
Alkaline Digest 2	271	311	257	286	271	279
Acid Soluble 3. Total P	245	259	204	244	244	239
Ester Phosphate	213	211	157	215	201	199
NAP by U.V. Absorption		83	69	91	79	81
P.P.2 (Inorganic P)	26.7	38.0	32.5	30.1	39.9	33.4
Ester Phosphate Residue	37.7	66.7	36.8	44.2	36.4	44.4

Table 33.

Analysis of Schneider Residue : Liver Nuclei from Normal Male Rats Fasted for 72 hours.

Results expressed as μg.P/100 mg. dry weight of nuclei.

	Preparation				
	43	52*	57	61	Mean
Fraction					
Insoluble 2	2.3	0.8	1.4	0.8	1.3
Alkaline Digest 2	346	257	333	305	310
Acid Soluble 3. Total P	285+	237	278	271	268
Ester Phosphate	228	217	228	221	224
NAP by U.V. Absorption			97	104	
P.P.2 (Inorganic P)	42.8	21.4	32.0	45.2	35.4
Ester Phosphate Residue	47.9	28.4	58.3	50.9	46.4

* Fasted for 48 hours

+ By calculation.

Table 34.

Analysis of Schneider Residue : Rat Liver Nuclei

Results expressed as μg.P/100 mg. dry weight of nuclei.

	Preparation	
	59 (Female)	45 (Male : Regenerating Liver)
Fraction		
Insoluble 2	1.0	0.8
Alkaline Digest 2	260	314
Acid Soluble 3. Total P.	217	282
Ester Phosphate	167	231*
NAP by U.V. Absorption	72.4	
P.P.2 (Inorganic P)	39.6	50.3
Ester Phosphate Residue	37.3	33.5

*By calculation.

Table 35.

Analysis of Schneider Residue : Summary.

Results expressed as μg.P/100 mg. dry weight of nuclei.

	Source of Nuclei				
	Fowl Erythro-cytes	Calf Thymus	Rabbit Liver	Rat (Fed) Liver	Rat (Fasted) Liver
Fraction					
Insoluble 2	1.6	1.1	1.0	1.4	1.3
Alkaline Digest 2	431	454	329	279	310
Acid Soluble 3. Total P	392	410	284	239	262
Ester Phos-phate	366	365	234	199	224
NAP by U.V. Absorption				81	
P.P.2 (Inor-ganic P)	15.5	22.8	16.9	33.4	35.4
Ester Phosphate Residue	43.5	49.6	45.6	44.4	46.4

3.3. Composition of Nuclei: Results expressed per nucleus.

Tables 36 to 43 show the average values for the content per nucleus of the phospholipid, RNA and DNA in the cell nuclei analysed and also the nuclear mass. The results are expressed as μg. x 10^{-6} per nucleus.

The results for fowl erythrocyte nuclei are given in Tables 36 and 37. The DNA content is fairly constant at about 2.5 μg. x 10^{-6} per nucleus. Phospholipid and RNA show a much greater variation. The fowl erythrocyte nucleus is very small and its dry weight is about 10×10^{-6} μg.

Only one sample of calf thymus nuclei was analysed in terms of number of nuclei present (Table 38). The DNA content is much greater (about 7.0 μg. x 10^{-6} per nucleus) than that of the fowl erythrocyte nucleus.

The rabbit liver nucleus (Table 39), like the fowl erythrocyte nucleus, shows a constant DNA content (of about 6.5 μg. x 10^{-6} per nucleus). RNA is also present in fairly constant amount.

It is of interest to know what changes take place in the composition of the cell nucleus under different physiological conditions and in Tables 40 and 41 the analysis of liver nuclei from fed and fasted rats is given.

The DNA content per nucleus is the same for fed and fasted animals. The RNA content of the liver nuclei of rats fasted for 72 hrs. is close to 2 μg. x 10^{-6} per nucleus, whereas in

Table 36.

Composition of Erythrocyte Nuclei from Normal Fowls.

Results expressed as μg. x 10^{-6}/nucleus.

	Preparation				
	FE 2	FE 8	FE 9	FE 10	Mean
Component					
Phospholipid	0.555	0.488	0.470	0.420	0.483
RNA by Phosphorus	0.194	0.305	0.417	0.082	0.250
" Pentose		0.346	0.153	0.114	0.203
" U.V. Absorption				0.082	
DNA by Phosphorus	2.39	2.80	2.20	2.46	2.46
" Diphenylamine		2.70	1.78	2.17	2.22
" U.V. Absorption				2.46	
Nuclear Mass	10.8	11.1	9.55	12.8	11.1

Table 37.

Composition of Erythrocyte Nuclei from Tumour-bearing Fowls.

Results expressed as μg. $\times 10^{-6}$/nucleus.

	Preparation			
	FE 4	FE 5	FE 7	Mean
Component				
Phospholipid	0.400	0.240	0.350	0.397
RNA by Phosphorus	0.073	0.196	0.147	0.139
" Pentose	0.111	0.155	0.139	0.135
DNA by Phosphorus	2.98	2.69	2.21	2.63
" Diphenylamine	2.43	2.99	2.10	2.51
Nuclear Mass	10.3	10.7	8.76	9.92

Table 38.

Composition of Calf Thymus Nuclei

Results expressed as μg. x 10^{-6}/nucleus.

	Preparation			
	CT 4a	CT 4b	CT 4c	Mean
Component				
Phospholipid	0.240	0.131	0.212	0.194
RNA by Phosphorus	0.496	1.34*	0.448	0.761
" Pentose	0.317	0.338	0.330	0.328
" U.V. Absorption		1.43*	0.476	
DNA by Phosphorus	7.43	7.04+	7.30	7.26
" Diphenylamine	6.52	7.01+	6.90	6.81
" U.V. Absorption		7.06+	7.13	7.10
Nuclear Mass	28.2	28.2	28.2	28.2

* Includes some DNA

+ Low due to loss of DNA to Acid Soluble 2 fraction.

Table 39.

Composition of Rabbit Liver Nuclei.

Results expressed as μ g. x 10^{-6}/nucleus.

	Preparation					
	RL 1	RL 2	RL 4	RL 5	RL 6	Mean
Component						
Phospholipid	0.198	0.623	1.55	1.19	0.628	0.698
RNA by Phosphorus	1.11	1.18	1.53	1.28	0.945	1.21
" Pentose	0.982	0.908	1.22	1.15	0.952	1.04
" U.V. Absorption				1.18	0.928	
DNA by Phosphorus	7.46	6.71	5.87	7.14	5.66	6.57
" Diphenylamine	8.08	6.67	6.42	7.60	5.19	6.79
" U.V.Absorption				7.16	5.31	
Nuclear Mass	41.4	42.1	50.5	46.1	41.7	44.4

Table 40.

Composition of Liver Nuclei from Rats on Stock Diet.

Results expressed as μg. x 10^{-6}/nucleus.

	Preparation					
	41	55	58	63	64	Mean
Component						
Phospholipid	4.10	1.04	2.74	0.77	2.05	2.14
RNA by Phosphorus	3.82	2.01	3.53	1.99	3.07	2.88
" Pentose	3.70	1.92	2.89	1.63	2.65	2.56
" U.V. Absorption		2.12	4.07	1.80	2.72	2.68
DNA by Phosphorus	10.0	9.42	10.7	9.07	9.05	9.65
" Diphenylamine	11.6	7.64	7.95	8.59	7.74	8.70
" U.V. Absorption		9.33	11.0	9.54	8.89	9.69
Nuclear Mass	101	66.2	93.5	62.9	73.5	79.4

Table 41.

Composition of Liver Nuclei from Rats Fasted for 72 hours.

Results expressed as μg. x 10^{-6}/nucleus.

	Preparation			
Component	43	57	61	Mean
Phospholipid	0.650	0.893	1.26	0.93
RNA by Phosphorus	2.20	2.08	2.11	2.13
" Pentose	1.88	1.71	1.92	1.84
" U.V.Absorption		1.64	2.13	1.89
DNA by Phosphorus	9.58	9.47	10.5	9.85
" Diphenylamine	9.40	9.45	9.96	9.27
" U.V.Absorption		9.21	10.3	9.76
Nuclear Mass	59.9	64.5	75.2	66.5

normal fed animals this value ranges from just under two to almost four. Such a variation is not unexpected in fed animals in this work since feeding was not controlled in any way although all animals were on the same stock diet.

Figure 5, in which phospholipid and RNA content are plotted against nuclear mass for each preparation, shows that there is a quantitative relationship between phospholipid, RNA and nuclear mass. The last is a very rough measure of the protein content of the nucleus.

In the experiments described above all the rats used were male albinos. In Table 42 the results of an analysis of liver nuclei from female albino rats (preparation 59) is shown and no significant difference is apparent. The DNA content of the liver nuclei of hooded males (preparation 70) is, in the one sample examined, lower than that of albino rats. Regenerating rat liver nuclei (albino male rats: preparation 45) show a slightly raised value for DNA. However only one preparation of nuclei has been examined and the difference cannot be claimed as significant. It is evident that the amount of RNA indicated by phosphorus analysis is much greater than that given by the reactions for pentose. This has not been observed to such a large extent in other preparations of rat liver nuclei.

The only other actively growing tissue studied was

Fig. 5. The RNA and Phospholipid Content of the Rat Liver Nucleus.

⊙ Male; Stock Diet. ● Male; 72hrs. Fast. ⊕ Female; Stock Diet.

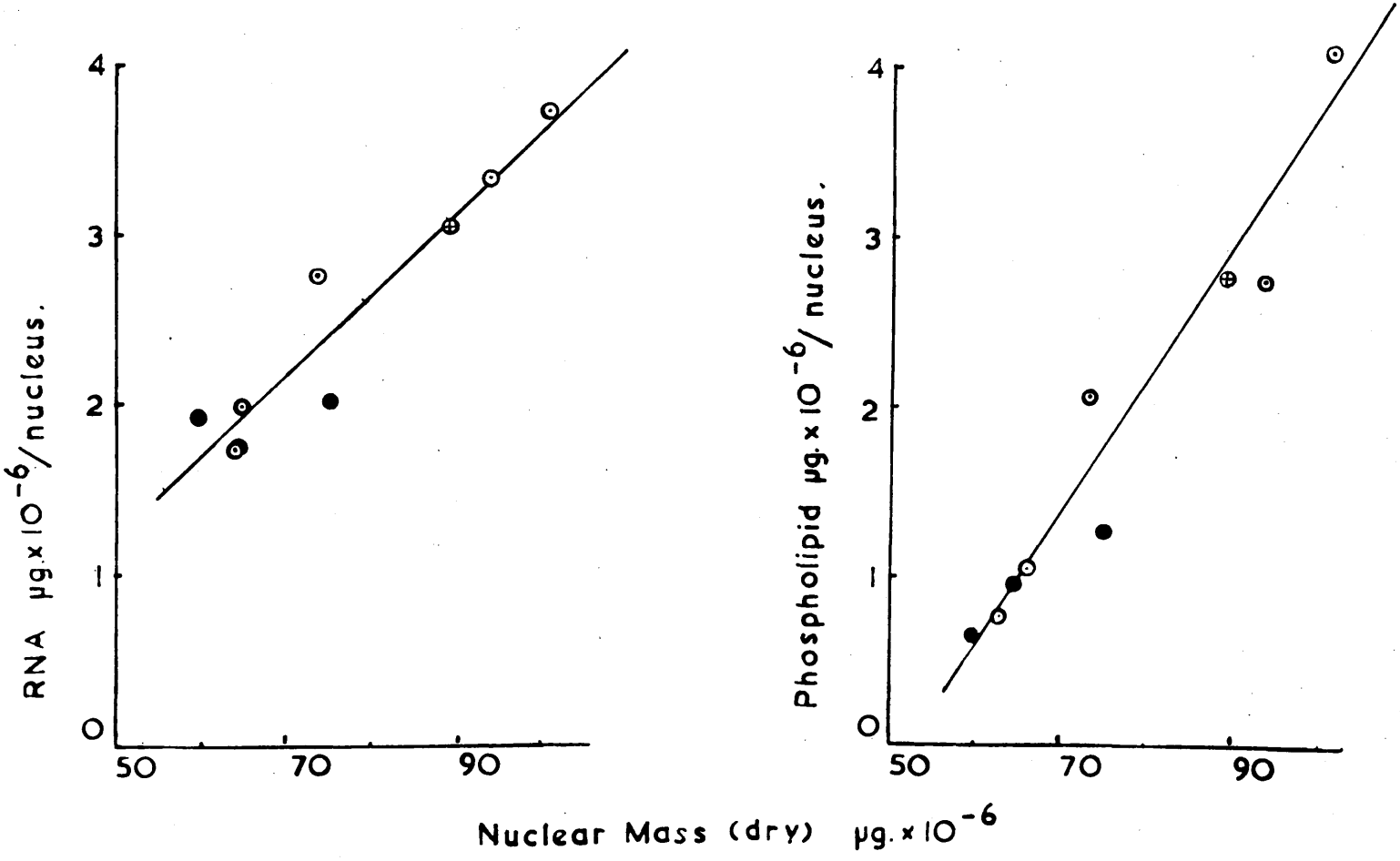


Table 42.

Composition of Rat Liver Nuclei.

Results expressed as μg. x 10^{-6}/nucleus.

Component	70 (Hooded; male)	59 (Albino; female)	45 (Albino; Regenerating liver)
Phospholipid	0.788	2.76	1.07
RNA by Phosphorus	2.52	3.34	4.05
" Pentose		2.96	2.81
" U.V.Absorption		3.01	
DNA by Phosphorus	8.51	11.1	11.4
" Diphenylamine		8.74	11.3
" U.V.Absorption		11.1	
Nuclear Mass		89.3	100

the GRCH 15 fowl tumour (see Table 43) which yields the surprising observation that the DNA content of the nucleus is approximately twice that of the fowl erythrocyte nucleus. The DNA content of the nuclei isolated from the liver of a fowl bearing a tumour is within the range found for the erythrocyte nuclei. The RNA content of the tumour nuclei is high. The large variation observed in different tumours is probably due in part to differences in their age.

Table 43. (Including Tables 44, 45 & 46).

Composition of Liver and Tumour Nuclei from Tumour-bearing Fowls.

Results expressed as μg. x 10^{-6}/nucleus.

Component	Tissue: Liver*	Tumour* 1	Tumour 2	Tumour 3+	Mean for Tumour
Phospholipid	0.191	10.3	1.54	1.21	4.35
RNA by Phosphorus	0.551	6.38	0.86**	2.34**	
" Pentose	0.477	7.50			
" U.V.Absorption	0.507	6.32			
DNA by Phosphorus	2.91	5.09	4.05	4.69	4.61
" Diphenylamine	2.64	4.30			
" U.V.Absorption	2.74	5.68			
Nuclear Mass	16.5	217			

* Tissues taken from same animal.

\+ Analysis carried out by Dr. I.Leslie of this department. (This preparation is considered to be of greater purity than samples 1 and 2).

** These figures are only approximate since the fraction analysed was Acid Soluble 2 which contains P.P.l.

3.4. Results of Experiments with Radioactive Phosphorus.

The extent of incorporation of ^{32}P into the various phosphorus-containing fractions of the cell nucleus during such a short period as two hours should give some idea of the relative metabolic turnover of these fractions and the specific activity of the different components studied is given in Tables 47 to 50.

The incorporation of ^{32}P into fowl erythrocyte nuclei was very small and the results are of only semi-quantitative significance owing to the very low counts obtained. However the "RNA" fraction of the erythrocyte nuclei had a specific activity generally more than 100 times that of the "DNA" fraction which was invariably very low.

Rabbit liver nuclei (Table 47) show a much greater incorporation of ^{32}P. The incorporation into DNA is low. However, the mean value for the ratio of the specific activity of "RNA" to that of "DNA" is 34, indicating a fairly rapid turnover of nuclear RNA. Phospholipid shows a specific activity of about ten times that of "DNA".

The inorganic phosphate present in the Acid Soluble 2 fraction (i.e., P.P.1) and probably derived from protein shows an extremely high specific activity except in one case, preparation RL 3, where a much smaller amount of starting material was available. P.P.1 has a higher specific

Table 47.

Specific Activity of the Fractions of Rabbit Liver Nuclei.

Results expressed as Counts/min./100 μg.P.

Fraction	Preparation RL 2	RL 3	RL 4	RL 5	RL 6
Acid Soluble 1	2070	1120	1560	2810	1060
Phospholipid	166	187	172	110	141
Insoluble 1	2400	0.00	0.00	0.00	0.00
Alkaline Digest 1	145	142	124	127	155
Acid Soluble 2	846	508	636	651	911
P.P.1	3380	373	3610	3810	3270
"RNA"	702	599	514	588	677
"DNA"	16.2	10.4	16.7	28.6	17.7
Schneider Extract	124		102	95	
Insoluble 2	380		0.00	1460	
Alkaline Digest 2	303		312	247	
Acid Soluble 3	367		391	257	
P.P.2	3850		2790	3200	
Ester Phosphate	94		102	123	
Ester Phosphate Residue	83		82	56.8	

activity than any other protein bound fraction and is therefore of great interest. As will be seen in Section 3.5, this fraction does not appear to be contaminated to any appreciable extent with phosphate originally present as inorganic phosphate in the isolated nuclei.

The Schneider Residue, generally presumed to contain only phosphoportein phosphorus, has a much lower specific activity than P.P.1, but the inorganic phosphate (P.P.2) liberated from this fraction on incubation in alkali has a specific activity comparable with that of P.P.1 and it is probable that these two fractions originate from the same source.

The phosphorus of the Schneider Residue which remains in organic combination (Ester Phosphate and Ester Phosphate Residue) has a low specific activity which is still, however, several times that of "DNA". The specific activity of the Schneider Extract is that expected from its RNA and DNA content.

Rat liver nuclei from fed and fasted animals (Tables 48 and 49) show a pattern of incorporation similar to that in rabbit liver nuclei, but the specific activities of the fractions relative to that of "DNA" are greater (with the exceptions of P.P.1 and P.P.2). There is no obvious difference in the specific activities of the various fractions obtained from the liver nuclei of fed and fasted rats.

Table 48.

Specific Activity of the Fractions of Liver Nuclei from Rats on Stock Diet.

Results expressed as Counts/min./100 μg.P.

	Preparation				
Fraction	41	55	58	63	64
Acid Soluble 1	2450	3240	3200	2370	2730
Phospholipid	822	411	820	775	1230
Insoluble 1	90	0.00	507	66	0.00
Alkaline Digest 1	333	295	392	319	374
Acid Soluble 2	1370	1570	1560	1980	1460
P.P.1	2930	2780	2900	3370	2890
"RNA"	1020	1410	1140	1560	1390
"DNA"	10.7	19.0	11.2	10.6	9.3
Schneider Extract	287	251	322	297	332
Insoluble 2	1870	0.00	253	1000	0.00
Alkaline Digest 2	574	582	752	563	717
Acid Soluble 3	611	598	953	658	829
P.P.2	2590	2400	3140	2410	2470
Ester Phosphate	297	191	369	216	279
Ester Phosphate Residue	146	184	275	157	216

Table 49.

Specific Activity of the Fractions of Liver Nuclei from Rats Fasted for 72 hours.

Results expressed as Counts/min./100 μg.P.

Fraction	Preparation 43	52*	57	61
Acid Soluble 1	1970	3440	1710	3070
Phospholipid	1220	1280	799	1640
Insoluble 1	0.00	0.00	92	179
Alkaline Digest 1	388	422	240	449
Acid Soluble 2	2330	1690	1420	2260
P.P.1	3110	4170	2460	3430
"RNA"	2110	1420	1350	2010
"DNA"	13.9	9.4	6.44	15.1
Schneider Extract	392	333	231	373
Insoluble 2	1580	667	382	75
Alkaline Digest 2	521	759	339	697
Acid Soluble 3	694	880	458	837
P.P.2	3070	3660	1790	2830
Ester Phosphate	201	227	145	208
Ester Phosphate Residue	157	191	80.4	201

*Rats fasted 48 hours.

Examination of female albino rats and hooded male rats (Table 50) yielded results similar to those obtained for male albino rats.

A striking difference is observed with nuclei prepared from rat liver which has been regenerating for 48 hours (Table 50). All fractions have a much lower specific activity (compared with those of normal liver nuclei) relative to that of "DNA". It is also apparent that all fractions of the Schneider Residue show specific activities decreased to as great an extent as those of "RNA" and P.P.1.

Table 50.

Specific Activity of the Fractions of Rat Liver Nuclei.

Results expressed as Counts/min./100 μg.P.

Fraction	Preparation 59 (Albino: female)	70 (Hooded: male)	45 (Albino Regenerating Liver)
Acid Soluble 1	2280	2040	297
Phospholipid	615	802	106
Insoluble 1	475	536	281
Alkaline Digest 1	310	313	86
Acid Soluble 2	1260	1430	349
P.P.1	2770	3340	506
"RNA"	1080	1320	244
"DNA"	11.8	13.7	16.4
Schneider Extract	257		96
Insoluble 2	284		180
Alkaline Digest 2	615		114
Acid Soluble 3	756		148
P.P.2	2390		516
Ester Phosphate	234		53.8
Ester Phosphate Residue	187		13.8

3.5. The Effect of Prolonged Washing with TCA on the Partition of Phosphorus in the Fractions of the Cell Nucleus, and the Specific Activity of the Fractions.

Since the samples of nuclei used in all the present experiments have been washed in dilute citric acid or 0.9% NaCl several times during their preparation and since the amount of acid soluble phosphorus removed by three washings with cold 10% TCA is small, it seems unlikely that sufficient inorganic phosphate, or other low molecular-weight phosphorus compound of high specific activity is present in the acid-washed nuclei to affect the specific activity of the fractions studied.

However, the effect of prolonged washing with 10% TCA on the quantity and specific activity of the phosphorus-containing fractions of the cell nucleus has been studied using nuclei from hooded rats injected with ^{32}P. One portion of the liver nuclei was dried from the frozen state (sample 1) and washed three times with cold 10% TCA. The remainder of the preparation, suspended in 0.2% citric acid, was divided into two equal portions. Sample 2 was washed with cold 10% TCA three times and sample 3 thirty times. A little radioactive material was always present in the later washings from sample 3. After the extraction of lipid the samples were submitted to the Schmidt & Thannhauser procedure. The results of the experiment are given in

Table 51.

When the nuclei are not dried before extraction with TCA (sample 2) more acid soluble material is extracted and its specific activity is lower. The other fractions do not seem to be greatly affected although P.P.1 is reduced in amount. Its specific activity, however, is little changed. On prolonged washing with TCA (sample 3) acid soluble 1, P.P.1 and "RNA" are increased in amount and "DNA" is decreased. (The comparison is made with the data for sample 2). The specific activity of P.P.1 is slightly lower and that of "RNA" decreased by more than 50%. Acid Soluble 1 and "DNA" show unchanged activities. The activity of Acid Soluble 1 (sample 3) is not much greater than that of "RNA" (sample 2) which suggests that the extra phosphorus present in Acid Soluble 1 may have its origin in RNA. The lower activity of "RNA" in sample 3 is probably due to a portion of the "DNA" fraction having become labile to alkali during the prolonged washing with TCA.

It should be emphasised that P.P.1 has maintained a high activity despite the drastic washing procedure. It therefore seems unlikely that the high activity of this fraction is due to contamination with highly active tissue inorganic phosphate which has failed to be removed during the extraction procedure.

Table 51.

Effect of Prolonged Washing with 10% TCA on the Phosphorus-containing Fractions of the Cells Nucleus (Rat (hooded) Liver). Results expressed as µg.P./100 mg. dry wt., and Specific Activity as Counts/min./100 µg.P.

	Total P in Fraction			Specific Activity of Fraction		
Sample*	1	2	3	1	2	3
Fraction						
Acid Soluble 1	22.5	103	140	2040	1530	1480
Phospholipid	47.7	69.6	72.5	802	795	779
Insoluble 1	1.70	2.65	3.57	536	362	132
Alkaline Digest 1	1800	1670	1570	313	329	229
Acid Soluble 2	400	423	501	1430	1260	690
P.P.1	22.4	13.4	33.6	3340	3090	2792
"RNA"	360	388	458	1320	1230	491
"DNA"	1300	1300	1070	13.7	18.2	18.6

*Sample 1. Dried from the frozen state and washed three times with 10% TCA.

" 2. Suspension of nuclei washed three times with 10% TCA.

" 3. Suspension of nuclei washed thirty times with 10% TCA.

3.6. The Effect of Reprecipitation on the Specific Activity of the "DNA" Fraction.

The "DNA" fraction has a low specific activity and is precipitated from a solution of much greater specific activity. It is therefore possible that it may be contaminated with small amounts of phosphorus of high activity although it is washed several times. It was therefore thought necessary to investigate the effect of reprecipitation on the "DNA" fraction. The results are presented in Tables 52 and 53.

In Table 52 the data were obtained using animals which had received the usual dose of ^{32}P, i.e., 10 μc./100 g. In spite of this the specific activity of the "DNA" fraction (R_1) was higher than usual. After one reprecipitation the specific activity was reduced by about 40% (R_2). If this fraction was brought to pH 6, the precipitate obtained (R_3) had a slightly greater activity and the supernatant (R_3S) showed a lower activity. Up to this point appreciable amounts of phosphorus were not lost. However, on precipitation of R_3S the loss of phosphorus was significant and the activity of the precipitate (R_4) was greater than that of R_3S. Resolution of R_4 and reprecipitation now decreased the activity while ionophoresis (see later) did not alter it.

It would appear that after the "DNA" fraction has been

Table 52.

Specific Activity of "DNA" Fraction from Rat Liver Nuclei on Reprecipitation.

Fraction		Number of Reprecipitations	Specific Activity of "DNA"
R1			33.0
R2		1	18.9
R3	Precipitated from R2 at pH6	2	22.8
R3S	Supernatant of above		14.5
R4	Precipitated from R3S with 10% TCA	2	18.0
R5	R4 reprecipitated	3	13.6
R5I	R4 submitted to ionophoresis		18.2

dissolved and reprecipitated once or twice there is a danger of serious degradation on further treatment. One reprecipitation appears to be most satisfactory as evidenced by the data of Table 53. The "DNA" fractions are those obtained in the ionophoresis experiments to be described in the next section and the animals used received 20 μc. P^{32}/100 g.

Table 53.

Effect of Reprecipitation on the Specific Activity of the "DNA" Fraction.

Specific Activity expressed as Counts/min./100 μg.P.

		Experiment				
		N_1	N_2	N_3	N_4	N_5
	Fraction No.					
"DNA"	R_1	49.6	31.9	-	-	-
	R_2 (Reprecipitated once)	28.6	20.9	29.3	29.8	12.8
	R_3 (Reprecipitated twice)	27.4	-	30.1	22.7	12.0
	R_4 (Reprecipitated thrice)	-	-	-	26.3	-

3.7. Separation of the Components of the Acid Soluble 2 Fraction of Isolated Nuclei by Ionophoresis.

It would obviously be of considerable interest to determine the composition of nuclear RNA and the turnover of the individual nucleotides. The Acid Soluble 2 fraction is a convenient starting material for studying these problems and the ionophoretic technique of Smellie & Davidson (1951) has been applied to this end.

Five components which absorb ultraviolet light have been detected and all contain phosphorus. Four of the components have ultraviolet absorption spectra corresponding to those of cytidilic acid, adenylic acid, guanylic acid and uridylic acid and occupy the positions taken up by the purified ribonucleotides (Smellie & Davidson, 1951).

Dr. G. Crosbie of this Department has hydrolysed the four components (obtained from rat liver nuclei) separately with perchloric acid and examined the hydrolysates for purine and pyrimidine bases by the chromatographic method of Markham & Smith (1949). It has been shown that each component contains the expected base and no other. We are therefore dealing with a nucleic acid qualitatively similar to yeast RNA.

The relative amounts of the four nucleotides and other components in the Acid Soluble 2 fraction of rat liver nuclei

is given in Table 54. Guanylic acid has been taken as a standard of reference since it is better separated from other components than is adenylic acid which is more often chosen.

The proportions of the four nucleotides is fairly constant. (Cytidylic acid in preparation N4 and adenylic acid in preparation N1 are exceptions). Cytidylic acid and guanylic acid are present in approximately equimolar amounts and uridylic and adenylic acids are less abundant. The purine to pyrimidine ratio is about 0.9.

The fifth component (Component 'X') which travels faster than the nucleotides contains a compound which absorbs generally over the range 2450A to 2750A. It also contains inorganic phosphate, which accounts for most of the phosphate present. The amount of organic phosphate was estimated by difference and is minute in preparations N4 and N5.

Recoveries of Acid Soluble 2 phosphorus were generally less than 90%. R.M.S. Smellie of this Department has recently shown that fraction Acid Soluble 2 from whole tissue contains at least one other unknown component in addition to 'X'. When this is taken into account recoveries of the order of 100% may be obtained.

The specific activities of the components just discussed are given in Table 55. During the period when the

Table 54.

Ionophoresis of the Acid Soluble 2 Fraction of Rat Liver Nuclei : Analysis.

Results expressed as moles P/mole. Guanylic acid P.

	Preparation				
	N1	N3	N4	N5	Average
Fraction					
Acid Soluble 2	4.43	4.18	4.60	4.37	4.40
Cytidylic Acid	1.04	1.07	0.800	0.957	0.967
Adenylic Acid	0.490	0.704	0.800	0.719	0.678
Guanylic Acid	1.00	1.00	1.00	1.00	1.00
Uridylic Acid	0.809	0.951	0.896	0.842	0.875
Component 'X'					
Total P	0.613	0.474	0.592	0.269	0.487
Inorganic P		0.439	0.576	0.237	0.413
Organic P (by difference)		0.035	0.016	0.032	0.028
Acid Soluble 2 (by addition)	3.95	4.20	4.09	3.79	4.01
% Recovery	89.3	101	88.9	86.5	91.1
Moles Purine	0.806	0.843	1.06	0.956	0.916
Moles Pyrimidine					

Table 55.

Ionophoresis of the Acid Soluble 2 Fraction of Rat Liver Nuclei : Specific Activities.

Specific activity expressed as counts/min./100 μg.P.

	Preparation				
	N1	N2	N3	N4	N5
Fraction					
Acid Soluble 2	2970	5060	2010	2770	935
Cytidylic Acid	2080		1570	2900	700
Adenylic Acid	2200		2210	2300	864
Guanylic Acid	1660		1350	1930	550
Uridylic Acid	2100		1680	2660	730
Component 'X'					
Total P	4580	8670	5170	6420	2250
Inorganic P		9970	5340	6020	1600
Organic P*		1210	1000	0	618
"DNA"	28.6	36.6	29.3	29.8	12.8

* Results of doubtful significance due to the small amount of phosphorus and radioactivity.

experiments employing ionophoresis were being carried out the samples of P^{32} as supplied by Harwell showed certain anomalies, the result being that the amount of ^{32}P injected into the animals was only approximately known. The specific activities of corresponding fractions are as a result very varied. For this reason the results presented in Table 55 have been recalculated assuming the activity of guanylic acid to be 1000 in all cases (Table 56). Of the four nucleotides adenylic acid invariably has the highest specific activity and guanylic acid the lowest. The two pyrimidine nucleotides have the same specific activity. The inorganic phosphate present in component 'X', like P.P.1 in the Schmidt & Thannhauser separation, shows a specific activity about twice that of the nucleotides. In preparation N5 the amounts of total phosphorus and inorganic phosphorus in X were low and the counts registered were also fairly low. The specific activities of component 'X', Total P and X Inorganic P are therefore less accurate than in the other preparations. The specific activities of the "DNA" fractions are included for comparison.

The results obtained by the ionophoresis technique indicate that the specific activity of the "RNA" fraction of the Schmidt & Thannhauser separation is close to that of RNA. A direct comparison of the results obtained by the two methods is given in Table 57.

Table 56.

Ionophoresis of the Acid Soluble 2 Fraction of Rat Liver Nuclei + Specific Activities.

Specific Activity expressed as Counts/min./100 μg.P.

Fraction	Preparation N1	N3	N4	N5	Average
Acid Soluble 2	1790	1480	1440	1700	1605
Acid Soluble 2 (by calculation)	1450	1520	1660	1470	
Cytidylic Acid	1250	1160	1510	1270	1300
Adenylic Acid	1330	1630	1710	1570	1560
Gyanylic Acid	1000	1000	1000	1000	1000
Uridylic Acid	1270	1240	1380	1330	1305
RNA (by calculation)	1230	1380	1270	1190	1270
Component 'X' Total P	2860	3830	3340	4090	3530
Inorganic P		3950	3130	2910	3330
Organic P*		743	0	1120	621
"DNA"	17.2	21.7	15.5	23.3	19.5

*Results of doubtful significance due to the small amount of phosphorus and radioactivity.

Table 57.

Comparison of the phosphorus-containing Components of Acid Soluble 2 obtained by the Schmidt & Thannauser Procedure and by Ionophoresis (Experiment N1).

Results expressed as moles.P/mole. Guanylic Acid.

Specific Activities expressed as counts/min./100 μgP : Guanylic acid = 1000.

	Sample 1 (Schmidt & Thannhauser)		Sample 2 (Ionophoresis)	
	Specific Activity	P	Specific Activity	P
Acid Soluble 2	1790	4.43		
Acid Soluble 2 (by calculation)			1450	3.95
"RNA"	1400	3.85		
RNA (sum of nucleotides)			1230	3.34
P.P.1	4330	0.62		
Component 'X' Total P			2860	0.61
"DNA"	17.2	30.3		

It is evident that organic phosphate has been lost on ionophoresis of Acid Soluble 2 since the total phosphorus of the nucleotides obtained by ionophoresis is less than that of the "RNA" fraction. The mean specific activity of the nucleotides is slightly less than that of "RNA".

All of the phosphorus of component 'X' is apparently accounted for by P.P.1 (inorganic P). But the small amount of organic phosphate in 'X' would account for the fact that the specific activity of component 'X' is lower than that of P.P.1.

The Acid Soluble 2 fraction of one preparation of rabbit liver nuclei has been submitted to ionophoresis. The results are presented in Table 58. The four nucleotides were identified by their absorption spectrum and are present in proportions similar to those in the RNA of rat liver nuclei. The phosphorus in component 'X' is almost all inorganic phosphate. The recovery of phosphorus was again less than 90%.

The results of the preceding ionophoresis experiments suggest that phosphorus compounds, as yet unaccounted for, are present in Acid Soluble 2. An attempt to decide this question was undertaken with nuclei from rat liver (Table 59), and with liver, tumour and erythrocytes of a tumour-bearing fowl (Tables 60 and 61) and calf thymus (Table 62).

Table 58.

Ionophoresis of the Acid Soluble 2 Fraction of Rabbit Liver Nuclei : Analysis.

Results expressed as moles.P/mole.Guanylic Acid P.

Fraction	
Acid Soluble 2	4.87
Cytidylic Acid	0.963
Adenylic Acid	0.735
Guanylic Acid	1.00
Uridylic Acid	1.03
'X' Total P	0.537
Inorganic P	0.500
Organic P (by difference)	0.037
Acid Soluble 2 (by addition)	4.265
% Recovery	87.6
Moles Purine	0.871
Moles Pyrimidine	

In the investigation of rat liver nuclei by ionophoresis Figs. 3 and 4 show ultraviolet light photographs of long and short ionophoresis runs side by side with radioautographs made from the same paper strips.

Consideration of the long run shows that the nucleotides detected by ultraviolet light absorption correspond in position on the strip to the highly radioactive areas which show up in the radioautograph. The radioautograph also reveals an active component located between adenylic and guanylic acids. Its specific activity is about half that of the nucleotides and the amount of phosphorus present is small.

In the short run component 'X' was cut out together with a small area behind it denoted component 'Y1'. The portion between 'Y1' and the nucleotide block has been named 'Y2'. The photograph of the short run in ultraviolet light (Fig.4) demonstrates 'X' quite conclusively but the radioautograph shows the high radioactivity of 'Y1' + 'X' to be located slightly behind the ultraviolet absorbing component. This suggests that the organic component of 'X' is moving faster than inorganic phosphate.

The amount of inorganic phosphate in 'Y1' + 'X' is about 50% greater than has been found previously (in 'X') and organic phosphate is also present in appreciable amount.

Table 59.

Ionophoresis of the Acid Soluble 2 Fraction of Rat Liver Nuclei.

Results expressed as moles. P/mole. Guanylic Acid P.

Fraction	(Long run) P	Specific activity	Fraction	(Short run) P	Specific activity
Acid Soluble 2	5.02	5560			
Cytidylic Acid	0.905	4365	Nucleotide block	4.21	
Adenylic Acid	0.745	5210	Component 'Y2'	0.247	1620
Intermediate	0.163	2200	Component 'Y1' + 'X' Total P	1.11	8080
Guanylic Acid	1.00	4180	Inorganic P	0.764	10910
Uridylic Acid	0.869	4150	Organic P (by difference)	0.346	1320
4 nucleotides + intermediate (by addition)	3.68		Acid Soluble 2 (by addition)	5.57	
$\frac{\text{Moles Purine}}{\text{Moles Pyrimidine}}$	0.98		% Recovery	111	

'Y1' is apparently an extension of 'X'.

The radioautograph (Fig.4) does not suggest the presence of radioactivity in area 'Y2'. However, a small amount of phosphorus is present having appreciable activity which is probably too diffusely distributed to be detected by the photographic film.

Thus two organic phosphates, hitherto undetected, have been located and together with the nucleotides and component 'X' (+ 'Y1') account for all the phosphorus of Acid Soluble 2. The high recovery in this experiment is due in part to traces of phosphorus in the paper.

In Tables 60 and 61 the results for nuclei from liver and tumour of a tumour-bearing fowl are given. The four ribonucleotides are present in the Acid Soluble 2 fraction and their relative amounts are about the same for both tissues. 'X' is also present and contains appreciable amounts of inorganic and organic phosphate. 'Y1' which was dealt with separately contains a small amount of phosphorus and is probably part of 'X' since the activity of the two components is almost the same in liver (Table 60).

The two new components (i.e., 'Y2' and the component between adenylic and guanylic acids) were also located but in small amount.

The animal used in the above investigation received

Table 60.

Ionophoresis of the Acid Soluble 2 Fraction of Liver Nuclei from a Tumour-bearing Fowl.

Results expressed as moles.P/mole.Guanylic Acid P.

Fraction (Long run)	P	Specific activity
Acid Soluble 2	3.38	792
Cytidylic Acid	0.635	251
Adenylic Acid	0.304	229
Intermediate	0.099	?
Guanylic Acid	1.00	268
Uridylic Acid	0.589	289
4 nucleotides + intermediate (by addition)	2.63	
Moles Purine		
Moles Pyrimidine	1.07	

Fraction (Short run)	P	Specific activity
Nucleotide block	2.93	339
Component 'Y2'	0.157	352
Component 'Y1'	0.091	3240
Component 'X' Total P	0.368	2850
Inorganic P	0.230	4520
Organic P (by difference)	0.138	373
Acid Soluble 2 (by addition)	3.55	
% Recovery	105	

Table 61.

Ionophoresis of the Acid Soluble 2 Fraction of Fowl Tumour Nuclei.

Results expressed as moles P/mole Guanylic Acid P.

Fraction	(Long run) P	Specific Activity	Fraction	(short run) P	Specific Activity
Acid Soluble 2	3.43	294			
Cytidylic Acid	0.722	100	Nucleotide block	3.06	165
Adenylic Acid	0.499	137	Component 'Y2'	0.141	59
Intermediate	0.109	?	Component 'Y1'	0.041	?
Guanylic Acid	1.00	113	Component 'X' Total P	0.478	1410
Uridylic Acid	0.632	128	Inorganic P	0.357	1415
4 nucleotides + intermediate (by addition)	2.96		Organic P (by difference)	0.122	236
Moles Purine / Moles Pyrimidine	1.11		Acid Soluble 2 (by addition)	3.72	
			% Recovery	108	

35 μc ^{32}P per 100 g. body weight 2 hours prior to killing. Despite the high dose all components, except 'X' (both tissues) and 'Y1' (liver), show very low specific activities. The "DNA" fractions have a relatively high specific activity: liver nuclei 48.0 and tumour nuclei 52.5.

The erythrocyte nuclei were also investigated but the amount of Acid Soluble 2 was too small for accurate estimations to be made. However the RNA present seemed to have a similar composition to that in the liver and tumour nuclei. It is also noteworthy that approximately 50% of the Acid Soluble 2 phosphorus was accounted for by 'Y1' and 'X' Total P and that both these, and particularly 'Y1', showed very high specific activities. The specific activity of the nucleotides was of the order of 400 and that of the "DNA" fraction was about 2.0.

The Acid Soluble 2 fraction of calf thymus nuclei showed certain anomalies and its investigation has yielded very interesting results (Table 62). The four ribonucleotides have again been identified and cytidylic acid and adenylic acid shown to be present in much smaller amount than the other two nucleotides. The component between adenylic and guanylic acids is present in an amount comparable with that of the nucleotides and 'X', which is again present, contains organic phosphate to the extent of about 65% of 'X' Total P.

Table 62.

Ionophoresis of the Acid Soluble 2 Fraction of Calf Thymus Nuclei : Analysis.

Results expressed as Moles P/Mole Guanylic Acid P.

Fraction (Long run)		Fraction (Short run)	
Acid Soluble 2	7.43	Nucleotide block	3.21
Cytidylic acid	0.467	DNA derivative	3.45
Adenylic Acid	0.436	Intermediate	0.131
Intermediate	0.368	Component 'X' Total P	0.187
Guanylic Acid	1.00	Inorganic P	0.064
Uridylic Acid	0.828	Organic P (by difference)	0.123
4 nucleotides + intermediate (by addition)	3.10	Acid Soluble 2 (by addition)	6.98
Moles Purine / Moles pyrimidine	1.11	% Recovery	93.9

Between 'X' and the nucleotide block (short run) a large spot was observed which strongly absorbed ultraviolet light. This component has an absorption peak at about 2600 A and was shown to contain adenine (plus a trace of guanine) by Dr. G. Crosbie of this department. It also contains deoxypentose (estimated by the diphenylamine reaction) and phosphorus in equimolar proportions. This DNA derivative represents almost half of the phosphorus of the Acid Soluble 2 fraction and probably accounts in large part for the discrepancies between pentose and phosphorus estimates of RNA noted earlier (Table 22). It is surprising that it moves faster than any of the ribonucleotides.

A further component which absorbs ultraviolet light strongly occurs near the starting point of the ionophoresis run. It contains guanine but no phosphorus or deoxypentose and may be free guanine derived from DNA.

Section 4. Discussion.

4.1. The Validity of Results obtained using Isolated Nuclei.

Two main problems must be faced when considering results obtained from a study of isolated nuclei. They are (1) the extent to which the nuclei isolated are representative of all the nuclei present in a tissue, and (2) the degree of alteration which the nuclei undergo on isolation.

A certain proportion of nuclei is broken during disruption of the cells in the Waring blendor (Schneider & Hogeboom 1951). This will, of course, affect the yield obtained. But it is also possible that the nuclei of one cell type (or the larger nuclei if the nuclear size varies) may be preferentially damaged and thus lost from the preparation. Such loss will affect both the yield and the quality of the preparation.

During the differential centrifugation of a tissue homogenate some muclei are inevitably lost if a clean preparation is desired. Should the nuclei vary in size and density there will be a tendency for the smaller and less dense nuclei to be lost. If this does, in fact, occur, the final preparation will not be representative of the nuclei of the tissue and its composition may therefore differ slightly from that of a wholly representative sample.

These possibilities should be kept in mind when the

results of analysis of isolated nuclei are considered.

The question of possible loss of DNA is of first importance in a consideration of the changes in composition undergone by nuclei during their preparation. Dounce (1943b) came to the conclusion that no DNA was lost from nuclei prepared at pH 3.8 to 4.0 although he presented no convincing evidence. Using the Feulgen staining technique Pollister & Leuchtenberger (1949) have shown that the nuclei of guinea-pig liver do not lose DNA when tissue slices are immersed in physiological saline at 5° for 3 hours. Ris & Mirsky (1949) have extended this type of experiment and shown that calf liver nuclei isolated in citric acid and the nuclei in tissue sections have the same DNA content when this is measured by the Feulgen technique. They have also shown that nuclei isolated in sucrose lose no DNA (Ris & Mirsky, 1949). The fact that nuclei prepared in citric acid at pH 4 and pH 6 have the same DNA content (Dounce et al., 1950) tends to confirm the above observations. Perhaps the most cogent proof that DNA is not lost to any appreciable extent from nuclei during their isolation is to be found in the agreement between several workers using a variety of methods, on the consistency of the DNA content per nucleus in several animal tissues. Since it has been proved with a reasonable degree of certainty that DNA is

not lost during the isolation of nuclei this component can be used as a standard by which to compare the composition of nuclei prepared by different methods.

Fairly convincing evidence that protein is lost during the isolation of nuclei in aqueous media has been presented recently by Dounce et al. (1950). The rat liver nuclei which they isolated in bulk by the Behren's technique contained about 5% DNA on a dry weight basis whereas those isolated in citric acid solution contained about 13% DNA. The former must therefore contain almost three times as much protein as the latter. Dounce et al. (1950) do not consider that this high protein content is due to cytoplasmic contamination but they make the unexpected observation that the RNA content of these nuclei is at least as high as the DNA content. However, their method of obtaining the RNA content is not above criticism.

Further evidence of loss of protein from isolated nuclei has been presented by Pollister & Leuchtenberger (1949). Using cytochemical techniques they have shown that the ratios of protein to DNA in the nuclei of sections of guinea-pig thymus and mouse liver are much higher than those reported for isolated nuclei. Although the figures which they give may be more reliable than those based on the analysis of isolated nuclei the methods used are not sufficiently well tested to be reliable. Greater significance

can be placed on their observation that 75% of the protein of the nucleus is lost when liver sections are immersed in physiological saline at 5° for 3 hours. Nuclei have not been prepared in this medium but the 'isolated chromosomes' of Mirsky & Ris were obtained from tissues homogenised in saline. The protein removed by the salt solution is more likely to be non-histone protein than histone. The latter may be removed in the isolation of nuclei by citric acid solutions (Dounce, 1943).

It can be said therefore that in all probability an appreciable amount of protein is lost from nuclei prepared in citric acid solutions. (Whether protein loss is incurred when nuclei are isolated in sucrose media is unknown.) DNA is evidently not removed from nuclei during isolation and so far the only evidence to suggest loss of RNA is that of Dounce et al. (1950) mentioned above.

4.2. The Phosphorus Compounds of the Cell Nucleus.

Analysis of the nuclei prepared in the present study has shown that 70-90% of the acid insoluble phosphorus of the cell nucleus is present in the DNA fraction and all the phosphorus in this fraction appears to be DNA phosphorus since ultraviolet absorption measurements are in close agreement with those obtained by phosphorus analysis. The results obtained for DNA by the diphenylamine reaction are also in

fairly good agreement with the latter although a greater scatter of values is apparent. Since the results of the diphenylamine reaction are dependent on the purine to pyrimidine ratio of the DNA under investigation it is doubtful if the use of thymus DNA as a standard throughout is justified. However, from recent work on the chromatographic separation of purines and pyrimidines, it appears that all samples of DNA from mammalian tissues so far examined have a purine to pyrimidine ratio of close to one (Chargaff, 1950).

The results for the DNA content of isolated cell nuclei (as % dry weight) presented here are lower than those of previous investigators (Table 14). This is probably due to a less serious loss of protein during the isolation of the nuclei.

The phospholipid content of erythrocyte and liver nuclei is appreciable and it accounts for as much as 6% of the phosphorus of the rat liver nucleus. The exact location of nuclear lipid is unknown although Claude (1949) states that the concentration in the nucleolus is high.

In the Schmidt & Thannhauser procedure all the phosphorus which does not originate in phospholipid and the DNA fraction is present in the Acid Soluble 2 fraction which has in the past been assumed to contain only ribomononucleotides and a small amount of inorganic phosphate presumably derived from phosphoprotein. The occurrence of the latter in whole

tissue was shown by Schmidt & Thannhauser (1945) and has been confirmed recently by Davidson, Frazer & Hutchison (1951).

The present investigations have shown that this fraction (P.P.1) occurs in small amounts in isolated nuclei (Davidson & McIndoe, 1949) and accounts for as much as 10% of the Acid Soluble 2 fraction of rat liver nuclei. Its occurrence in isolated nuclei has also been noted by Marshak & Calvet (1950) and by Marshak (1951). There is as yet no absolute proof that this inorganic phosphate is derived from true phosphoprotein but it has been known for a long time that treatment with dilute alkali liberates inorganic phosphate from the phosphoproteins of milk and eggs (Plimmer & Bayliss, 1906).

According to current opinion the organic phosphate of the Acid Soluble 2 fraction should represent ribomononucleotides. However the presence of RNA in the cell nucleus has hitherto not been conclusively proved although the presence of pentose as judged by the orcinol and phloroglucinol reactions has been strongly suggestive.

The present investigation provides conclusive evidence of the occurrence of nuclear RNA. The good agreement between the results of pentose estimations of U.V. absorption measurements and of estimations of organic phosphorus confirms the presence of RNA, although pentose estimates often give lower

values particularly in fowl erythrocyte and calf thymus nuclei. That the material corresponding to RNA in the nucleus does, in fact, have a nucleotide structure, has now also been proved.

The ionophoresis technique of Smellie & Davidson (1951) applied to the Acid Soluble 2 fraction of isolated nuclei affords a means of investigating the "nuclear RNA", qualitatively and quantitatively, without the necessity of attempting to isolate it. Five components which absorb ultraviolet light and contain phosphorus can be separated. Four of these are the nucleotides, cytidylic acid, adenylic acid, guanylic acid and uridylic acid and it is therefore evident that the nucleus contains a nucleic acid of the conventional pentose type.

The composition of the RNA of the nuclei examined is presented in Table 63 together with the corresponding figures recently published by other workers after the present investigation was completed. Some results on the composition of whole tissue RNA are included for comparison. With the exception of calf thymus RNA, nuclear RNA seems to be characterised by having relatively more uriclylic acid and adenylic acid than does whole tissue RNA. This is certainly true of rat liver where several samples of nuclear RNA were examined.

Marshak (1951), in his examination of calf thymus nuclei, obtained his figures for the ribonucleotides by

Table 63.

The Composition of the RNA of Whole Tissue and Isolated Nuclei

(Molar ratios of the nucleotides, guanylic acid taken as 1.00).

Tissue	Source	Cytidylic Acid	Adenylic Acid	Guanylic Acid	Uridylic Acid	Purine / Pyrimidine	Reference
Rat liver	Whole Tissue	0.94	0.58	1.00	0.66	0.99	Smellie & Davidson (1951)
" "	Nuclei	0.97	0.68	1.00	0.88	0.91	Present work
" "	Nuclei	1.39	0.89	1.00	0.95	0.81	Elson & Chargaff (1951)
Rabbit liver	Whole Tissue	0.87	0.61	1.00	0.75	1.00	Smellie & Davidson (1951)
" "	Nuclei	0.96	0.74	1.00	1.03	0.87	Present work
Calf Thymus	Cytoplasm	0.77	0.53	1.00	0.48	1.22	Marshak (1951)
" "	Nuclei	0.10	1.14	1.00	0.19	7.4	" "
" "	"	0.47	0.44	1.00	0.83	1.11	Present work
GRCH 15 Tumour	Whole Tissue	0.60	0.30	1.00	0.27	1.59	Beale, Harris & Roe (1950)
" " "	Nuclei	0.72	0.50	1.00	0.63	1.11	Present work
Liver from tumour-bearing fowl	Nuclei	0.66	0.30	1.00	0.59	1.07	Present work

estimating the individual bases in an Acid Soluble 2 fraction after hydrolysis. The remarkably high proportions of adenine and guanine observed in calf thymus nuclear RNA by Marshak are probably due to the presence of purine derivatives of DNA in his Acid Soluble 2 fraction. Such derivatives have been observed in the present study of calf thymus nuclei.

The fifth component obtained in the ionophoresis run, component 'X', contains inorganic phosphate (corresponding to P.P.1) and an organic phosphate. The organic component which shows a general absorption of ultra violet light, apparently runs slightly faster than the inorganic phosphate. This phenomenon is difficult to explain but is at present under investigation in this Department.

The phosphorus of Acid Soluble 2 not accounted for by the nucleotides and 'X' has been located in components 'Y1' and 'Y2' and in a component occurring between adenylic and guanylic acids. 'Y1' would appear to be largely if not wholly inorganic phosphate (i.e., an extension of Inorganic P of component X). The nature of the other two components is not yet known.

Two other substances occur in the Acid Soluble 2 fraction of calf thymus nuclei. One is a nucleotide derivative of DNA, which is apparently adenine deoxyribonucleotide. However, it travels faster than any of the ribonucleotides on ionophoresis (and much faster than adenylic acid). The other is free guanine apparently also derived from DNA. Calf thymus DNA is thus degraded by mild alkaline hydrolysis in a way not observed for other types of DNA.

These purine derivatives of DNA largely account for the marked discrepancy between estimates of "RNA" by pentose

and by organic phosphate and U.V. absorption carried out on fraction Acid Soluble 2.

Thus the Acid Soluble 2 fraction of isolated nuclei contains three unidentified phosphorus containing compounds which, together with the ribonucleotides and inorganic phosphate, account for all the phosphorus present except in calf thymus nuclei where the remainder is accounted for by DNA derivatives.

4.3. The RNA content of the Nucleus.

Few data are avilable with which to compare the present results on the RNA content of the nucleus. Villela (1949) found that snake erythrocyte nuclei contained less than 1% RNA on a dry weight basis. Present results indicate that the RNA content of the fowl erythrocyte nucleus is fairly low (about 1.5% dry weight). The figure for calf thymus nuclei is similar and the RNA to DNA ratio of about 0.7 is comparable with that given by Euler & Hahn (1946). About 15% of the total nucleic acid of rabbit liver nuclei and 22% of the nucleic acid of rat liver nuclei is present as RNA. These figures are similar to those published by Nash (1949) and Bergstrand et al. (1948) for the nuclei of mouse liver and rat liver respectively.

Values for the RNA content per nucleus are similar to those published by Vendrely & Vendrely (1948). A depletion of the RNA of the rat liver nucleus apparently occurs when

the animals are fasted. A loss of phospholipid is also indicated and since the dry weight of the nucleus is decreased considerably protein is presumably lost too. Unpublished work of Klein, Kurnick & Klein (1950) confirms a loss of RNA and protein from the nuclei of fasted animals.

Results of the estimation of RNA based on the reactions for pentose will be influenced by the purine to pyrimidine ratio of the RNA under examination. The value for yeast RNA used as a standard was about 1.15 whereas that for the RNA of rabbit and rat liver nuclei was about 0.9. Values for RNA by pentose for these nuclei will therefore be about 10% low.

4.4. The DNA Content of the Nucleus*.

The investigations of Boivin, Vendrely & Vendrely (1948) and Vendrely & Vendrely (1948 and 1949b) showed that the DNA content of the nucleus of somatic cells was constant for different tissues of the same species and equal to twice the content of the sperm. (If we presume that DNA is a necessary component of the chromosomes this is in agreement with genetic theory.) Davidson, Leslie, Smellie & Thompson (1950) have similarly shown that the DNA content of the nucleus is the same in several tissues of the adult and

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* For a review see Davidson & Leslie (1950).

embryo fowl. The work of the Vendrelys has also demonstrated that this constancy prevails throughout several mammalian species.

The present experiments were designed to investigate the degree of constancy of the DNA content (per nucleus) of the nuclei of any one tissue of a single species and a reasonable number of samples of nuclei from fowl erythrocytes, rabbit liver and rat liver have been examined.

The nuclei of these tissues show a constant DNA content within the limits of experimental error. The value obtained here for fowl erythrocyte nuclei (about 2.5×10^{-6} μg. DNA per nucleus) is in agreement with those of Vendrely & Vendrely (1949a and 1950), Mirsky & Ris (1949) and Davidson, Leslie, Smellie & Thompson (1950) and that for rabbit liver nuclei (about 6.5×10^{-6} μg. DNA per nucleus) is in agreement with those of Vendrely & Vendrely (1949b) and Klein, Kurnick & Klein (1950).

The value of about 10×10^{-6} μg. DNA per nucleus found for rat liver nuclei is much higher than has been found for mammalian tissues generally by Vendrely & Vendrely (1948 and 1949b) and for other rat tissues (Davidson, Leslie, Smellie & Thompson, 1950 and Leuchtenberger, Vendrely & Vendrely, 1951). It is, however, in agreement with figures obtained by other workers (Davidson, Leslie,

Smellie & Thompson, 1950; Dounce et al., 1950; Price, Miller, Miller & Weber, 1950). Cunningham, Griffin & Luck (1950) and Leuchtenberger et al. (1951) have also observed high values. These high figures are generally explained by the fact that polyploidy is common in the rodent liver (Jacobj, 1925). Evidence in support of this comes from the cytochemical observations of Ris & Mirsky (1949), Pasteel & Lison (1950) and Leuchtenberger et al. (1951) who have shown that on the basis of DNA content three nuclear classes corresponding to diploid, tetraploid and octaploid nuclei, occur in rat liver and similar results have been obtained for mouse liver by Swift (1950).

With the exceptions of the tissues showing polyploidy the cytochemical investigations of Ris & Mirsky (1949), Leuchtenberger et al. (1951) and Pasteel & Lison (1950) have generally confirmed the view that all nuclei in a tissue have the same DNA content, although Pasteel & Lison (1950) conclude, from their study of the rat, that the relationship between the DNA content of the somatic nuclei and that of the spermatozoa is not so simple as Vendrely & Vendrely (1948) suppose.

Few studies have been made of the DNA content of the nucleus under different physiological conditions. Fasting and protein deficiency are the only conditions so far investigated. It has been shown that the total DNA content

of rat liver remains constant on fasting (Davidson, 1947) and the present work on rat liver has shown more conclusively that the DNA content of the nucleus is unchanged after fasting for a period of 72 hours. A similar conclusion was reached by Mandel, Jacob & Mandel (1949, 1950) who kept rats on a protein free diet for long periods and observed no change in the number of nuclei or in the DNA content of the liver.

The value which has been obtained for the DNA content of the nuclei of regenerating liver is slightly higher than those found in normal liver. Since only one figure is available little significance can be attached to this result but it should be noted that Price & Laird (1950) have found that the nuclei of rat liver in the very early stages of regeneration have a DNA content which is almost twice that of normal liver nuclei. The DNA content falls almost to normal within 24 hours.

In recent years some information has been obtained on the DNA content of tumour nuclei. Figures presented here show that the DNA content of the nuclei of the GRCH 15 tumour is about twice that of the nuclei of erythrocytes from normal and tumour bearing birds. The figure for erythrocytes is the same as that for other tissues of the fowl (Davidson, Leslie, Smellie & Thompson, 1950).

Goldberg, Klein & Klein (1950) and Klein & Klein (1950)

have observed that the cells of the Ehrlich ascites tumour contain twice the amount of DNA present in the cells of normal mouse tissues but that the Krebs sarcoma nucleus did not show an enhanced DNA content. Higher values than normal were indicated by Cunningham, Griffin & Luck (1950) for rat hepatoma produced by feeding 3'-methyl-4-dimethylaminoazobenzene but not for the tumour produced by acetylaminofluorene. The nuclei of liver showing precancerous changes also had a normal DNA content. Similar work by Price, Miller, Miller & Weber (1950) has shown the DNA content of nuclei from normal rat liver and from the livers of animals treated with aminoazo dyes to be the same and Mark & Ris (1949) found that the DNA content of the nuclei of hepatoma and cholangioma was the same as that of normal rat liver nuclei.

Greenstein (1947) has drawn attention to the great similarity between tumours of different types. In the work just described we have a differentiation of tumours into two types: those in which the DNA content of the nuclei is the same as that in normal tissues and those in which it is approximately twice the normal value.

Observations on the spleen of leukaemic mice (Paterman, Alfin-Slater & Larace, 1949; Arnesen, Goldsmith & Dulaney, 1949) have revealed no increase in the DNA content

of the nuclei compared with those of normal spleen, although considerable increase in the RNA content of the nuclei was observed. No difference between the DNA content of bone marrow cells from normal human subjects and those with leukaemia was demonstrated by Davidson, Leslie & White (1950 and 1951). The value was however significantly raised in cases of pernicious anaemia.

4.5. The Turnover of the Constituents of the Cell Nucleus.

The incorporation of ^{32}P into the nuclear phospholipids of rabbit and rat liver is fairly rapid. From work carried out in this Department on whole tissue (Davidson, Frazer & Hutchison, 1951) and that presented here it appears that the rate of incorporation of ^{32}P into the phospholipids of the nucleus is comparable with that into whole tissue phospholipids.

Hevesy (1946) has reported the specific activity of the phospholipid of rat liver nuclei to range from 50% to 80% of that of whole liver phospholipid 2 hours after administration of ^{32}P. Similar results have been obtained by Barnum & Huseby (1950) for rat liver and by Ada (1949) who studied the nuclear and cytoplasmic fractions of rabbit liver. However Marshak & Calvet (1949) observed that the incorporation of ^{32}P into nuclear phospholipid was more rapid than into the cytoplasmic phospholipid of rabbit liver.

Since Marshak in 1948 postulated the presence in the cell nucleus of a nucleic acid of high metabolit activity, interest in the metabolism of nuclear RNA has increased.

The specific activity (2 hours after injection of ^{32}P) of the organic phosphorus of the Acid Soluble 2 fraction of rabbit and rat liver nuclei is very high (Davidson & McIndoe, 1949) indicating a rapid incorporation of ^{32}P into nuclear RNA. The presence of small amounts of organic phosphate in Acid Soluble 2 which is not bound as ribonucleotide will influence the specific activity measurements to a slight extent. However, the high specific activity of the individual mononucleotides confirms the rapid turnover of nuclear RNA in rat liver.

Comparison of the cytoplasmic and nuclear ribonucleotides of rat liver (Davidson, McIndoe & Smellie, 1951) has shown the nuclear RNA to have a specific activity about twenty times that of cytoplasmic RNA. The results obtained on the incorporation of isotopes into whole tissue RNA may therefore be misleading since a high proportion of ^{32}P incorporated into tissue RNA will, in the first few hours after injection, be located in the relatively small amount of RNA present in the nucleus. The greater part of the tissue RNA will have a lower turnover rate than is indicated by most isotope investigations.

The work of Jeener & Szafarz (1950) and of Barnum & Huseby (1950) has confirmed the high activity of nuclear RNA in rat liver and Marshak & Calvet (1949) have obtained similar results using rabbit liver. That ^{32}P is incorporated into nuclear RNA almost as rapidly as it appears in the tissue inorganic phosphate or acid soluble phosphorus fractions is indicated by the experiments of Barnum & Huseby (1950) and Marshak & Calvet (1949).

The specific activity of the nuclear ribonucleotides of rat liver decreases in the order adenylic acid, cytidylic and uridylic acids and guanylic acid. This pattern is different from that observed for RNA in the cytoplasmic fractions (Davidson, McIndoe & Smellie, 1951). Precise determination of the relative rates of incorporation of ^{32}P into the individual nucleotides would, however, require the construction of a specific activity-time curve. In a study of the incorporation of ^{32}P into rabbit liver, Marshak & Vogel (1950) demonstrated that the nucleotides of nuclear RNA had the same specific activity and claim that this activity is maintained over a period of 3 to 24 hours after injection of ^{32}P. This is a contradiction of the earlier observation of Marshak & Calvet (1949) that the specific activity of rabbit liver nuclear RNA decreases rapidly four hours after ^{32}P injection.

Jeener & Szafarz (1950) have suggested from the results of their isotopic experiments that RNA is synthesised in the nucleus and then passes into the cytoplasm. Barnum & Huseby (1950) analysed their own results mathematically and favour the independent synthesis of nuclear and cytoplasmic RNA.

Evidence that cells synthesising nucleic acid produce RNA first in the nucleolus is presented by Lagerstedt (1949) and by Barr & Bertram (1949). Since much of the nuclear RNA is located in the nucleolus, these results are in accord with the higher turnover rate of nuclear RNA than of cytoplasmic RNA which, according to Hyden (1943), Caspersson (1947) and Lagerstedt (1949), first appears near the nuclear membrane. In these observations we have circumstantial evidence for the transfer of nuclear RNA to the cytoplasm during RNA synthesis. It should be noted, however, that nuclear and cytoplasmic RNA, at least in rat liver, do not have the same purine and pyrimidine content so that the view that cytoplasmic RNA originates by simple transfer from the nucleus would appear to be questionable.

The "phosphoprotein" phosphorus fraction (P.P.1, P.P.2 and 'X' Inorganic P) of the cell nucleus shows an extremely high specific activity. In whole tissue this has been shown to be due in part to contaminating ^{32}P not removed in the acid extraction (Davidson, Frazer & Hutchison, 1951),

but the contamination of isolated nuclei is less likely to be appreciable since the acid soluble material in the preparations is very small in amount and since exhaustive washing of the nuclei with ice-cold TCA apparently affects the chemical properties of both RNA and DNA without reducing the activity of P.P.1 to any great extent. It has also been observed that "phosphoprotein" phosphorus has a higher activity in the nucleus than in the cytoplasm (Davidson, McIndoe & Smellie, 1951) and since inorganic phosphate is much more abundant in the cytoplasm than in isolated nuclei, this is further evidence against the view that contamination by tissue inorganic phosphate is responsible for the high activity of P.P.1 (or 'X' Inorganic P). From the results obtained it appears that ^{32}P is incorporated into the "phosphoprotein" of the nucleus at an even greater rate than into the nuclear RNA and this is confirmed in the ionophoresis experiments. Marshak & Calvet (1949) have made similar observations on rabbit liver nuclei. The "phosphoprotein" fraction has also been noted in rat liver nuclei by Jeener & Szafarz (1950) who, however, have not considered it in detail.

The organic phosphates recently detected in the Acid Soluble 2 fraction in small amount have specific activities between those of nuclear RNA and of DNA but an accurate

estimate of these values could not be made because of the small amount of material present.

The metabolic stability of the phosphorus moiety of DNA has been known for several years through studies with ^{32}P. This is confirmed in the present studies on the DNA fraction of isolated nuclei. Marshak & Calvet (1949) and Barnum & Huseby (1950) have also found the specific activity of DNA fractions from isolated nuclei to be very low. The latter authors found a nine-fold increase in 24 hours indicating a very low turnover rate. Working with ^{15}N-labelled adenine Furst, Roll & Brown (1950) have shown the incorporation of adenine into the DNA purines of rat liver to be extremely low and to be of the same order as the rate of mitosis. In regenerating rat liver incorporation into the DNA purines was greatly enhanced as had previously been found in earlier work with ^{32}P. In the one experiment carried out here on regenerating liver in the rat the specific activity of the DNA fraction of the nuclei was two or three times greater (in comparison with that of the other phosphorus compounds) than in normal liver. The general lower level of specific activities observed in this experiment compared with that found in studies of normal liver may well be due to impaired blood supply resulting in a low concentration of ^{32}P in the liver tissue.

The results of Volkin & Carter (1951) suggest that the incorporation of ^{32}P into the DNA of normal and regenerating liver is similar. However, work in which [^{14}C - 2] glycine (Le Page & Heidelberger, 1951) and [^{14}C - 2 orotic] acid (Wilson 1951) were used, provides evidence for the increased turnover of DNA in regenerating liver.

Recent investigations employing [^{14}C - 2] glycine and ^{14}C-formate to study the metabolism of the nucleic acids indicate that DNA may not be as inactive as the work with ^{32}P has suggested. Le Page & Heidelberger (1951) and Heidelberger & Le Page (1951) using [^{14}C - 2] glycine have shown that incorporation of ^{14}C into the bases of DNA is approximately equal to that into RNA of rat tissues. Their DNA, however, was contaminated with RNA which may well have been of nuclear origin and have contained a larger proportion of isotope than whole tissue RNA (cf. studies with ^{32}P). The observed incorporation of ^{14}C into DNA may therefore have been too high. But the work of Elwyn & Sprinson (1950) confirms the rapid incorporation of glycine into DNA.

Totter, Volkin & Carter (1951) using ^{14}C formate also suggest that the metabolic turnover of the DNA bases is considerable, but the incorporation of [^{14}C - 2] orotic acid into nucleic acid pyrimidines seems to be much lower for DNA than for RNA (Wilson, 1951). Appreciable incorporation of cytidine labelled with ^{15}N into the pyrimidines of

DNA has been observed (Hammarsten, Reichard & Saluste, 1950).

The degree of incorporation of isotopes into the nucleic acid bases seems to vary greatly with the particular precursor chosen as the isotope carrier. However the work with ^{32}P on the one hand and labelled glycine and formate on the other suggest that the DNA bases are metabolised independently of the phosphorus.

Summary.

1. An investigation has been made of the phosphorus compounds of isolated cell nuclei from fowl erythrocytes, calf thymus, fowl, rabbit and rat liver and the GRCH 15 tumour of the fowl. The results have been expressed both in terms of dry weight of nuclear material and as an absolute amount per single nucleus.

2. Of the total phosphorus present in the cell nucleus 70-90% is present as deoxyribonucleic acid (DNA).

3. The DNA content per nucleus of several samples of nuclei from fowl erythrocytes, and rabbit and rat liver is constant for each nuclear type. This constancy is maintained in rat liver even after a 72 hour fast during which there is a decrease in the RNA, phospholipid and protein content of the nucleus.

4. The nuclei of the GRCH 15 tumour have approximately twice the DNA content (per nucleus) of the nuclei of the normal somatic tissues of the fowl.

5. The observation that DNA phosphorus is metabolically almost inactive has been confirmed in experiments in which ^{32}P was used.

6. The presence of ribonucleic acid (RNA) in the cell

nucleus has been established by analytical methods. By means of ionophoresis nuclear RNA has been shown to contain the same four nucleotides as RNA from other sources although the relative amounts of the nucleotides are different from those of cytoplasmic RNA.

7. Nuclear RNA incorporates ^{32}P very rapidly in rat liver and the individual nucleotides show different incorporation rates.

8. The presence of phospholipid in the cell nucleus has been confirmed and its turnover shown to be appreciable.

9. "Phosphoprotein" has been detected in the nucleus in small amount and its phosphorus shown to be metabolically highly active.

10. At least two further protein-bound phosphorus containing compounds have been observed in small amount. Their nature is not yet known.

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