Phosphorus Compounds in the Cell

4. THE INCORPORATION OF RADIOACTIVE PHOSPHORUS INTO LIVER CELL FRACTIONS

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In part 3 of this series (Davidson & Smellie, 1952*b*) a method was described for the accurate determination of the specific activities of the individual ribonucleotides of the ribonucleic acid (RNA) of the tissues of animals which had received radioactive phosphorus. This method has now been applied to the RNA of cell nuclei and of the various morphological fractions of the cell cytoplasm. Preliminary notes on this work have already been published (Davidson, McIndoe, & Smellie, 1951; Smellie & McIndoe, 1952) and a full account is given here.

METHODS

Biological. Albino rats from the departmental colony and chinchilla rabbits bred in the Department, were used in these experiments. Both received 50 μc. ^{32}P/100 g. body weight as carrier-free inorganic phosphate by intramuscular injection.

When rabbits were used, samples of blood (5 ml.) were withdrawn from the ear vein 2 hr. after injection of isotope in order that the specific activity of the blood inorganic phosphate might be determined.

Rats were killed by exsanguination under ether anaesthesia and the rabbits by cervical dislocation. The livers were perfused with cold 0·9% (w/v) NaCl solution, dissected out and chilled. The liver tissue was finely minced with scissors and pulped in 0·25 M-sucrose either in a Potter-Elvehjem (1936) homogenizer or, where larger amounts of material were involved, in an M.S.E. Nelco blendor (Measuring and Scientific Equipment Ltd., London, S.W. 1) with the blades replaced by a paddle. The duration of this process was controlled by repeated microscopical examinations to ensure maximal rupture of cells with minimal breakdown of nuclei. Rabbit liver proved more resistant to disintegration than did rat liver. The suspension was first strained through gauze and centrifuged at 600*g* to remove nuclei and any unbroken cells. The deposit was washed once with an equal volume of 0·25 M-sucrose and the first supernatant and washings combined.

Nuclei were isolated either from the sediment obtained in this way after blending at low speed in citric acid or from a separate sample of the original liver tissue. The citric acid method as described by McIndoe & Davidson (1952) was employed in either case.

The cytoplasmic supernatant material from the sucrose suspension was routinely examined microscopically to verify the absence of nuclei. Subsequent fractionation followed the pattern of Schneider (1948), the fields employed for the sedimentation of the various fractions being as follows: mitochondria, 8500 *g* for 10 min.; microsomes, 25 000 *g* for 60 min. The supernatant fluid after removal of the microsomes constituted the cell sap. Centrifugation was carried out in an International or M.S.E. refrigerated centrifuge fitted with a high-speed attachment.

In agreement with the observation of Muntwyler, Seifter & Harkness (1950) and of Schneider & Hogeboom (1951) it was found that the top layer of the mitochondrial sediment obtained as described above was loosely packed by comparison with the main bulk. It was decanted along with the supernatant fluid for further fractionation. The quality of the mitochondrial preparation was checked from time to time by adding Janus Green B to a concentration of 1/100 000 in the original suspension. The stained mitochondrial sediment when incubated at 38° for 5 min. turned red. This property enabled it to be distinguished sharply from microsomal material (Potter, Recknagel & Hurlbert, 1951).

Electron microscopy. Samples of the preparations of mitochondria and microsomes were fixed with OsO_4 in 0·25 M-sucrose for 15 min. and spun down. This material was washed twice with distilled water to remove all traces of sucrose. The resultant fixed suspensions were prepared for examination in the electron microscope by allowing a small drop of a suitable dilution of the specimen to evaporate to dryness on a prepared specimen mount, a standard polyvinyl formal ('Formvar') film on a Philips slotted film support. The uninterrupted area of 0·2 mm.^{2} available for examination in the electron microscope is much greater than that obtainable with most other types of mount, and in this case it was possible to ensure that a completely representative field was chosen for photography. The specimens were shadowcast with palladium (Williams & Wyckoff, 1945) at an angle of 15°. The shadow length in the micrographs is thus approximately 3·5 times the particle height.

Chemical. The main bulk of the mitochondrial, microsomal and cell sap fractions was suspended in distilled water and treated with 0·5 vol. 30% (w/v) trichoroacetic acid (TCA). A sample of whole cytoplasm was likewise treated with TCA and from this acid extract inorganic phosphate

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Table 1. *Specific activities in counts/min./100 μg. P of the nucleotides of the RNA from the cytoplasmic fractions and nuclei of the liver tissue of a group of rats which had received* ^{32}P *2 hr. before being killed*

	Whole cytoplasm	Mitochondria	Microsomes	Cell sap	Nuclei
Adenylic acid	383	289	189	650	5920
Guanylic acid	201	182	109	303	4800
Cytidylic acid	360	215	141	575	5000
Uridylic acid	286	232	119	435	4870
DNA fraction	—	—	—	—	140

was precipitated as described by Davidson, Frazer & Hutchison (1951) and used for the determination of specific activity.

The material precipitated by TCA from all four fractions was washed twice with 10% (w/v) TCA and treated with lipid solvents. From the protein residue RNA was extracted by 'procedure 2' of Davidson & Smellie (1952*b*), hydrolysed with alkali and separated into the component ribonucleotides by ionophoresis on paper (Davidson & Smellie, 1952*a*). Portions of the nucleic acid hydrolysates were in some cases also used for determination of the base ratios as described in the following paper (Crosbie, Smellie & Davidson, 1953).

Nuclear RNA was obtained as a solution of mixed ribonucleotides by the modification of the Schmidt & Thannhauser (1945) procedure described by McIndoe & Davidson (1952). These were also separated by ionophoresis on paper.

The general methods employed in the assay of radioactive materials were those of Davidson, Frazer & Hutchison (1951).

The results of radioactivity measurements are expressed in terms of counts/min./100 μg. P. In the experiments on rabbits it was felt desirable to have a common denominator to which all specific activities might be related, especially when the results obtained at different time intervals were being compared. The specific activity of the blood inorganic phosphate at 2 hr. was accordingly determined and used for the calculation of the relative specific activity (R.S.A.). Where groups of rats were employed this method was not practicable. In such cases, when it was necessary to compare one series of experiments with another at the same time interval, results were expressed relatively to the tissue inorganic phosphate at the time of killing.

In the experiments on regenerating rat liver partial hepatectomy was carried out by the method of Higgins & Anderson (1931).

RESULTS

Electron micrographs of mitochondria and microsomes illustrated in Pl. 1 indicate that each of these fractions is relatively homogeneous in the morphological sense, and that the two fractions differ markedly from each other in the size of the component particles. The diameters of the mitochondria range from 410 to 860 mμ. with a mean value of 550 ± 90 mμ. and those of the microsomes from 10 to 200 mμ. There is no appreciable overlap in the sizes of the two groups. The mitochondria are, on the whole, circular or nearly circular in outline, and the individual particles throw large shadows indicating that the mitochondria themselves are nearly spherical. The particle density after osmic acid fixation is such that there is little transmission of an 80 kV. electron beam and there is no suggestion of internal structure. In contrast, the photographs of microsomes show a rather wide array of particle sizes and the individual particles are in every case irregular in outline. The large particles invariably throw short shadows and the impression gained from the micrograph is that the material consists of varying sizes of aggregates of a component of about 10 mμ. particle diameter.

The specific activities of the ribonucleotides from the RNA of various parts of the rat-liver cell are shown in Table 1 for an experiment of 2 hr. duration. It is clear that the RNA of the nucleus is very much more active than that of any of the cytoplasmic fractions. Of these fractions themselves the cell sap gives values about twice as high as those obtained from the granules. We have invariably found in many experiments of this duration in rats and rabbits that the ribonucleotides from the microsomes are slightly less active than those from the mitochondria. The figures for the whole cytoplasm, as might be expected, are intermediate between those for microsomes and cell sap.

There appears to be no great difference in specific activity between the four ribonucleotides from any one source, but adenylic acid is generally slightly higher and guanylic acid slightly lower in activity than the pyrimidine nucleotides.

In Table 2 the results of an experiment of 4 hr. duration are given in terms of R.S.A. with tissue inorganic phosphate taken as 1000. The pattern for the control animals which were submitted to the sham operation is the same as for the normal animals shown in Table 1, although the values for mitochondria and microsomes lie very close together. The regenerating livers, however, present some differences from the controls. The nuclear ribonucleotides again show very high activities which are about twice those found in the control animals. The activities of the ribonucleotides from the whole cytoplasm are also raised to values about 3 or 4 times those in the controls. The rise in the activity of the ribonucleotides in the whole cytoplasm is reflected in each of the three fractions. Of the cytoplasmic fractions the cell sap still gives the highest figures while mitochondria and microsomes are almost equal. The nucleotides in each fraction again show only slight differences among themselves, adenylic acid as usual showing the highest values.

Table 2. *Specific activities (counts/min./100 μg. P) relative to tissue inorganic phosphate as 1000, of ribonucleotides from cytoplasmic and nuclear RNA and of DNA from the livers of partially hepatectomized rats (R) (regenerating 26 hr.), of sham operated rats (C) and of weanling rats (W)*

(^{32}P was administered 4 hr. before killing.)

	Whole cytoplasm			Mitochondria			Microsomes			Cell sap			Nuclei		
	R	C	W	R	C	W	R	C	W	R	C	W	R	C	W
Phospholipin	402	207	480	366	213	416	422	242	511	396	278	478	370	211	385
Adenylic acid	83	24	39	75	19	29	74	16	33	98	35	49	590	361	370
Guanylic acid	69	16	27	51	13	22	64	12	21	80	23	33	606	318	349
Cytidylic acid	74	23	37	69	16	25	59	14	20	94	41	50	590	320	338
Uridylic acid	72	21	33	67	19	21	62	12	20	90	31	38	702	308	380
DNA fraction	—	—	—	—	—	—	—	—	—	—	—	—	43	3	14

Table 3. *Specific activities (counts/min./100 μg. P), relative to the adult blood inorganic phosphate at 2 hr. as 100 000, of phospholipins, of ribonucleotides from cytoplasmic and nuclear RNA and of DNA from the livers of rabbits 4 hr. after receiving ^{32}P*

(N, normal female; M, pregnant female; F, foetus.)

	Whole cytoplasm			Mitochondria			Microsomes			Cell sap			Nuclei		
	N	M	F	N	M	F	N	M	F	N	M	F	N	M	F
Phospholipin	1860	2730	1750	1430	—	—	1710	—	—	1710	—	—	1480	3250	1910
Adenylic acid	485	559	1090	341	361	389	212	126	628	495	861	1520	5990	9160	8970
Guanylic acid	450	256	940	308	276	277	162	134	540	302	432	1160	4890	8660	7440
Cytidylic acid	323	423	855	252	290	324	151	213	558	438	610	1230	5670	7960	7580
Uridylic acid	456	572	1020	307	340	328	148	147	696	438	830	1422	4530	8820	10250
DNA fraction	—	—	—	—	—	—	—	—	—	—	—	—	273	600	3260
Tissue inorganic phosphate	33600	73100	42900	—	—	—	—	—	—	—	—	—	—	—	—

Figures for the specific activities of the phospholipins are also given in Table 2. There is no great difference between nuclear and cytoplasmic material, but the activity in all fractions is greater in the regenerating liver than in the controls.

As might be expected, the deoxyribonucleic acid (DNA) in the nuclei of the control livers shows a very low activity. In the regenerating livers, on the other hand, in which cell division is active the specific activity is increased about 15-fold.

In the weanling rats, which were all about 50 g. body weight, the specific activity of the DNA, as might be expected for growing tissue, is about 5 times greater than in the controls, giving a value intermediate between those for resting and regenerating adult livers. Values for phospholipin are of the same order as those for regenerating liver. The activities of the ribonucleotides follow the same general pattern as in the sham-operated rats but are in all cases slightly higher.

The results of an experiment on maternal and foetal liver obtained from a pregnant rabbit are compared in Table 3 with those from a normal female animal. These results are expressed as R.S.A. in terms of the adult-blood inorganic phosphate at 2 hr. as 100000, but they may also be expressed relatively to the tissue inorganic phosphate of the liver concerned by using the data given in the last line of the table.

The pattern found for the normal rabbit is similar to that obtained for the rat. In the pregnant animal the specific activities of the nuclear ribonucleotides and phospholipins are increased as compared with the normal animal. This also holds for the ribonucleotides of the cell sap, but not for the granules. The figure for the specific activity of DNA is also slightly raised.

Conditions in the foetus, however, are very different. The specific activity of the DNA is high; phospholipins both in nuclei and cytoplasm show slightly lower values than are given by maternal liver, while nuclear ribonucleotides give figures of the same order as are found in the maternal tissue.

The specific activities for the ribonucleotides of whole cytoplasm are much higher than those found in normal or maternal liver, and these differences are due to the high values found in the cell sap and microsomes, especially the latter. Figures for mitochondria are of the same order as those for maternal liver.

Whereas in normal and maternal rabbit liver (and also in normal, regenerating and weanling rat liver) the specific activity of the ribonucleotides from the mitochondria was invariably of the same order as, or slightly higher than, that of the ribonucleotides from the microsomes, in foetal liver the situation is reversed and the microsomes give values considerably higher than those for the mitochondria,

although appreciably lower than those found in the cell sap.

If the results are expressed relatively to the tissue inorganic phosphate there is no change in the relationships between the fractions from any one type of liver, but the relationships of the various types of liver to each other are slightly altered. For example, the activities of the nuclear ribonucleotides from normal and maternal liver become closely similar while those for the foetal liver are much higher. In the cytoplasm the normal figures become higher than the maternal while those for the foetus remain higher than both.

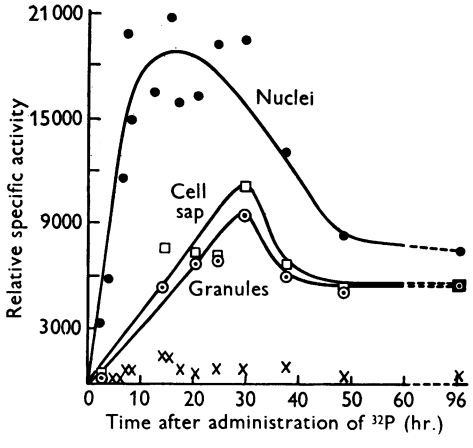


Fig. 1. Specific activity (counts/min./100 μg. P) relative to the specific activity of blood inorganic phosphate, 2 hr. after administration of the isotope, of the cytidylic acid from the RNA of cytoplasmic granules (⊙), cell sap (□) and nuclei (●) of rabbit-liver tissue at varying intervals after the administration of ^{32}P. The mitochondria and microsomes gave values so close together that they have been combined as 'granules'. The crosses represent the specific activities of DNA.

The results which have so far been described were obtained from animals which were killed 2 or 4 hr. after administration of ^{32}P, but experiments have also been carried out with rabbits in which time intervals ranging from 2 to 96 hr. were employed with the results shown in Fig. 1.

These curves were obtained from the cytidylic acid of the nuclear and cytoplasmic RNA's. The other three nucleotides give similar patterns. At time intervals greater than 4 hr. the figures for mitochondria and microsomes lie so close to each other that these two fractions have had to be represented together as 'granules'. Figures for cell sap are consistently slightly higher than those for granules and reach a maximum value at about the same time (approx. 30 hr.) after administration. Values for nuclear cytidylic acid rise very steeply to a maximum of about twice that found for cytoplasmic nucleotides. Although the figures show a fairly wide scatter, it is clear that the maximum occurs earlier (about 15 hr.) for nuclear cytidylic acid than for cytoplasmic material.

Even at 96 hr. the figures for nuclear and cytoplasmic material remain surprisingly high, but the value for nuclear cytidylic acid is still higher than that for cell sap or granules.

The specific activity of the DNA is at all time intervals low and shows no evidence of a progressive rise with time.

DISCUSSION

In any process of fractionation of cellular components one of the first considerations must be the technique used for the breakdown of the cells. The Waring type of blender produces such powerful shearing forces as to cause disruption of cellular components (in particular nuclei) in a sucrose medium. The Potter-Elvehjem (1936) glass homogenizer is more suitable for the purpose but does not readily handle large amounts of material. In addition, a small quantity of powdered glass is produced which can prove troublesome in the subsequent analysis. We have therefore used a Potter-Elvehjem type of homogenizer with a Perspex pestle (Potter, 1949). For large amounts of material the rotating paddle has proved to give good disruption of cells in sucrose without undue destruction of nuclei.

Microscopic examination of these suspensions has shown that a very large number of free and apparently undamaged nuclei are still present, together with a few whole cells. The presence of these whole cells is inevitable unless disintegration is considerably prolonged with a resultant breakdown of large numbers of nuclei. Confirmation of the absence of large numbers of broken nuclei lies in the fact that the DNA content of the whole cytoplasm, after removal of the nuclear fraction, has been found to be negligible. Since the ratio RNA:DNA in the nucleus is not more than 1:5 it is reasonable to suppose that there is little contamination of cytoplasmic RNA with RNA derived from broken nuclei, although it is not impossible that some RNA may have been leached out of the intact nuclei during the initial stages of fractionation.

It is generally accepted that differential centrifugation of suspensions of liver pulp in sucrose yields particles of two main types, large particles corresponding to the mitochondria and small particles corresponding to the microsomes (Schneider & Hogeboom, 1951), although it has been claimed by Chantrenne (1947) that the range of heterogeneity is much greater than can be accounted for by this simple classification. Chantrenne (1947), however, used a buffered saline solution in which the mitochondria and microsomes tend to agglutinate

(Schneider & Hogeboom, 1951), and this might possibly explain the spectrum of particles he observed.

Using suspensions of liver pulp in 0·88 M-sucrose, Novikoff, Podber, Ryan & Noe (1952) separated eight fractions, three of which were essentially mitochondrial, two were mixtures of mitochondria and microsomes and three were essentially microsomal. They concluded that the fundamental products of differential centrifugation are mitochondria and large and small microsomes. The latter would presumably correspond to the microsomes of different sizes shown in Pl. 1. In our experience these microsomes, of whatever size, failed to give the characteristic reaction with Janus Green B which was observed in the mitochondria.

The evidence obtained from electron microscope examination (Pl. 1) is strongly in support of the view that there are two varieties of cytoplasmic particles, the mitochondria which appear as very well-defined spherical granules and the microsomes which are less homogeneous in appearance. On the basis of structure alone it is possible to identify the components from each fraction in artificially mixed samples since the large structures contributed by the microsomal preparations, as shown in the lower picture in Pl. 1, are clearly loose aggregates of smaller particles, whereas the granules contributed by the mitochondrial preparations are distinct spherical entities. As with all morphological work with fixed material in the electron microscope, the possibility of fixation artifacts cannot be completely eliminated, but care was taken in these experiments to ensure that both fractions were given the same treatment during fixation. It is therefore reasonable to assume that the structural differences observed represent real distinctions between the two fractions. For this reason, however, the study of the detailed morphology of the large granules themselves is made difficult in the present state of knowledge of fixative action on nucleoprotein-containing structures. The observations made on partially osmolysed granules would support those reported by Claude & Fullam (1945) and by Mühlethaler, Müller & Zollinger (1950).

The determination of the specific activities of protein-bound phosphorus compounds is well known to present serious difficulties owing to the presence of highly labelled non-nucleotide materials (Davidson, Frazer & Hutchison, 1951). The method of ionophoresis, which has been employed in these experiments, is known to overcome these difficulties and to provide true values for the specific activities of the ribonucleotides (Davidson & Smellie, 1952*b*). From the results in Tables 1–3, it is clear that the incorporation of ^{32}P into RNA is of the same order in all four nucleotides from any one subcellular component, although the activity of adenylic acid tends to be slightly higher and of guanylic acid slightly lower than those of the pyrimidine nucleotides. This is in agreement with the observations of Volkin & Carter (1951). The high figures for uridylic acid quoted by Davidson, McIndoe, & Smellie (1951) are due to the presence of other components of high activity which have been described by Davidson & Smellie (1952*b*). The presence of these constituents is almost certainly the cause of the high figures for uridylic acid mentioned by Hultin, Slautterback & Wessel (1951).

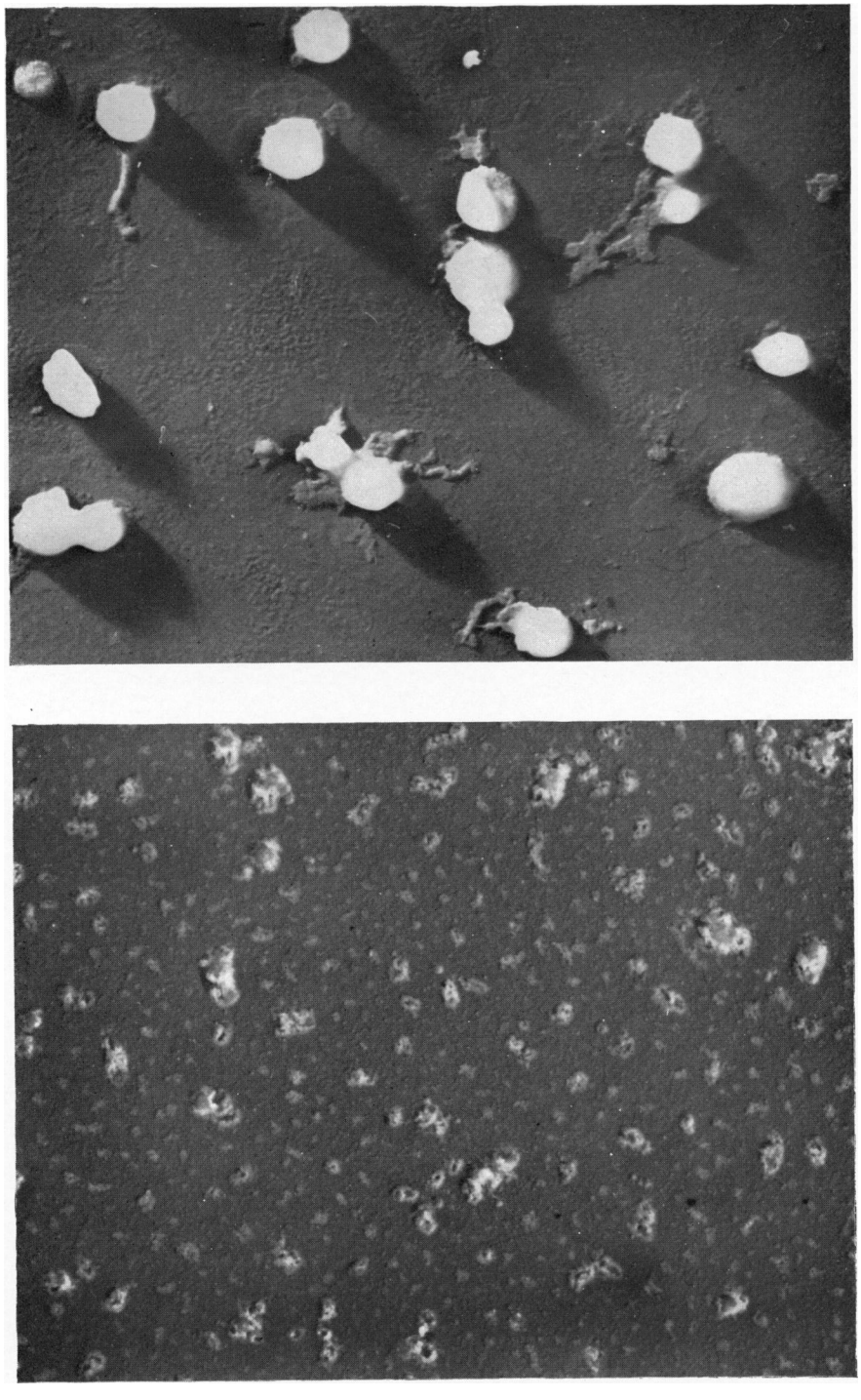
The very high specific activity of the ribonucleotides from nuclear RNA is consistent with the results of other authors whose work has recently been reviewed by McIndoe & Davidson (1952).

We have invariably found with time intervals of 2 or 4 hr. in normal rat or rabbit liver that the specific activity of the ribonucleotides from the microsomes is of the same order as, or slightly lower than, that of the ribonucleotides from the mitochondria both in sucrose and in saline suspensions. Figures for the cell sap are always higher than those for the granules. The possibility cannot be excluded that this high figure is due in part at least to the presence in this fraction of some RNA which has been leached out of the nuclei.

The differences between the specific activities for the ribonucleotides of the mitochondria and microsomes at short time intervals is small. With increasing time the specific activities of the nucleotides from mitochondria and microsomes are greatly increased without a proportionate increase in the difference between them, so that this difference tends to become negligible.

Marshak & Calvet (1949), using rabbit liver, found a higher value for the specific activity of small cytoplasmic granules than for large granules at varying time intervals between 1 and 73 hr. after administration of ^{32}P, but their granules were obtained from a suspension of liver pulp in 5 % (w/v) citric acid. Specific activity measurements were made on the bulk nucleotide fraction obtained by the method of Schmidt & Thannhauser (1945). This fraction is known to contain contaminating phosphorus compounds of high specific activity (Davidson, Frazer & Hutchison, 1951; Davidson & Smellie, 1952*b*).

Jeener & Szafarz (1950), using the livers of rats 2 hr. after administration of ^{32}P, found a higher specific activity in their small granule fraction (pellet, $6 \times 10^4 g$ for 60 min.) than in their large granule fraction (pellet, $1{\cdot}3 \times 10^4 g$ for 10 min.). In mouse embryo this situation was reversed. The RNA of the supernatant fluid invariably showed higher activity than any of the granules. It should be emphasized that Jeener & Szafarz (1950) disintegrated their tissue in a Waring type of homogenizer and centrifuged it in phosphate buffer under centrifugal fields considerably higher than those employed



Electron micrographs of cytoplasmic particles prepared by differential centrifugation from a suspension of rat-liver tissue in 0·25 M-sucrose. Magnification ×15 000. Shadowed at 15° with palladium. Upper picture, mitochondria; lower picture, microsomes.

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by us. It is therefore scarcely justifiable to compare their results with ours, but it seems possible that the high centrifugal fields which they employ yield a large-granule fraction which is a mixture of mitochondria and microsomes and a microsome fraction which contains some of the larger molecules of ribonucleoprotein from the cell sap. This tendency would be emphasized by their use of phosphate buffer in place of sucrose as the suspending medium.

Barnum & Huseby (1950), using saline suspensions of the livers of mice which had received ^{32}P, found no appreciable difference between the R.S.A. of isolated RNA from mitochondria and microsomes. They did, however, observe that the specific activity of the phospholipin in mitochondria was significantly lower than that of the microsomes. A similar observation has been recorded by Ada (1949) and is in agreement with our own results (Table 2).

In two types of liver tissue in which cell division is occurring, regenerating rat liver and the livers of weanling rats, the same general pattern appears as is found in normal rat liver although the specific activities are in general higher than for normal tissue (Table 2). High values for the specific activities of RNA in regenerating rat liver have also been recorded by Khesin (1951) and Johnson & Albert (1952).

In the maternal rabbit liver, as compared with the normal, the high specific activity of the ribonucleotides is particularly noticeable in the cell sap while the values for the granules are almost unchanged (Table 3). The foetal liver is unique in that the activity in the microsomes is increased disproportionately so that the value exceeds that of the mitochondria. This is the reverse of the results obtained by Jeener & Szafarz (1950) for mouse embryo.

The specific activity of the DNA is increased in weanling rat liver, a moderately rapidly growing tissue, and greatly increased in rat liver regenerating 26 hr. after partial hepatectomy (Table 2). At this stage cell division is very active. The ratios of the specific activities of RNA from whole cytoplasm to nuclear DNA are about 7:1 for normal rat liver and 1·7:1 for regenerating liver. These ratios agree well with figures previously quoted for whole isolated nucleic acids by Davidson (1947). The corresponding figure for normal rabbit liver is 1·6:1. The approximately twofold increase in specific activity of DNA from pregnant-rabbit liver over that for normal rabbit liver (Table 3) is in agreement with the results of Kelly, Payne, White & Jones (1951) for rat liver.

In the foetal rabbit liver the specific activity of the DNA is increased about tenfold in comparison with the adult tissue, while that of the RNA is approximately doubled. The result is that the activity of RNA is considerably lower than that of the DNA (ratio 0·3:1). Ratios less than unity have already been recorded by Davidson (1947). In the sea-urchin embryo Villee, Lowens, Gordon, Leonard & Rich (1949), using ^{32}P, and Abrams (1951), using [^{14}C]glycine, have shown that the specific activity of DNA is much higher than that of RNA, without distinguishing the intracellular location of the RNA.

The results shown in Fig. 1 emphasize the differences between nuclear and cytoplasmic RNA. The nuclear material not only incorporates ^{32}P more readily but the uptake reaches a maximum earlier than in cytoplasmic RNA. Our experiments on rabbits show a maximum incorporation into nuclear RNA about 15 hr. after administration. The corresponding maximum for the nuclear RNA of mouse liver incorporating ^{32}P was found by Barnum & Huseby (1950) to be about 2 hr. after administration, while Hurlbert & Potter (1952) found the maximum incorporation of radioactive orotic acid by nuclear RNA in the rat to occur about 4 hr. after administration.

Marshak & Calvet (1949) quote a few figures indicating maximum incorporation of ^{32}P into rabbit-liver nuclear RNA between 2 and 12 hr. after administration.

Mauritzen, Roy & Stedman (1952), discussing nuclear RNA in a recent paper, state that they are unable to decide whether the RNA of isolated cell nuclei is a true nuclear component or an impurity adsorbed from the cytoplasm. Our results make it clear that the nucleotides of rabbit-liver nuclear RNA incorporate ^{32}P about 6 times more rapidly than do the nucleotides of cytoplasmic RNA from any fraction. These results confirm the discrete nature of nuclear RNA.

Jeener & Szafarz (1950), on the basis of their experiments with radioactive phosphorus, have suggested that RNA after passing from the nucleus into the cytoplasm appears first in a non-sedimentable form and that it is then built up into the microsomes and so into the mitochondria. If this hypothesis were correct three separate specific activity/time curves would be expected with maxima in the order: cell sap, microsomes and mitochondria. Our experiments on ^{32}P incorporation into the RNA's of rabbit liver at different time intervals are not altogether compatible with this proposal since the activity/time curves of the RNA's from the three cytoplasmic fractions are closely similar.

Our results do not in any way preclude the possibility that nuclear RNA serves as the ultimate precursor of cytoplasmic RNA's, although the activity/time relationships which we observed (Fig. 1) are not consistent with a process of simple diffusion from the nucleus into the cytoplasm. Such a process moreover would be expected to yield cytoplasmic RNA of the same molar composition

as that of nuclear RNA whereas marked differences have been observed (McIndoe & Davidson, 1952; Crosbie *et al.* 1953). The specific activity/time relationships of the RNA's from the cytoplasmic particles suggest that they are being synthesized at the same rate from a common precursor. The differences between the cytoplasmic particles and the cell sap are sufficiently small to be explained on the basis of permeability of the particles or of slight contamination of the cell sap with nuclear RNA.

SUMMARY

1. Using the technique of differential centrifugation in 0·25 M-sucrose, rat- and rabbit-liver cell cytoplasm have been separated into two particulate fractions and a non-sedimentable supernatant fluid corresponding to the cell sap. Isolated cell nuclei have also been prepared.

2. The particulate fractions correspond to the mitochondria and microsomes and have been characterized by Janus Green staining and by electron microscopy which has shown them to consist of two independent populations of particles.

3. The incorporation of ^{32}P into the phospholipins and ribonucleic acid (RNA) of the isolated nuclei and three cytoplasmic fractions and into the deoxyribonucleic acid (DNA) of the isolated nuclei has been studied in normal, regenerating and weanling rat liver and in normal, maternal and foetal rabbit liver. In all cases the most rapid incorporation was found in nuclear RNA. In the cytoplasm the cell sap invariably showed a higher activity than the granules. In foetal rabbit liver the microsomes showed a higher activity than the mitochondria. In all the other forms of liver tissue studied the activity of the microsomes was always of the same order as or slightly lower than that of the mitochondria.

4. The incorporation of ^{32}P into RNA in the various morphological fractions of normal rabbit liver has been determined at various time intervals. Nuclear RNA showed maximum incorporation about 15 hr. after administration whereas the three cytoplasmic fractions all showed maximum incorporation at about 30 hr.

5. Incorporation of ^{32}P into DNA in resting liver tissue was very low. In rat liver, regenerating after partial hepatectomy, the activity of the DNA was greatly increased, and in foetal rabbit liver was raised to a value much higher than that of the cytoplasmic RNA.

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