The Incorporation of Radioactive Phosphorus into the Nucleic Acids of Different Rabbit Tissues

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In a previous paper (Smellie, McIndoe, Logan, Davidson & Dawson, 1953) we have discussed the incorporation of radioactive phosphorus (^{32}P) into the deoxyribonucleic acid (DNA) of the cell nucleus of rabbit liver and into the ribonucleic acid of the nucleus (nRNA) and of the various cytoplasmic fractions (cRNA) at different times after administration of the isotope. As might be expected in a non-proliferating tissue, the incorporation into the DNA was only slight. On the other hand, the incorporation into the cRNA was relatively great and at a maximum at about 30 hr. after administration, while that into the nRNA was even greater and at a maximum at about 18 hr. It is known from the early work of Hevesy & Ottesen (1943) and of Andreasen & Ottesen (1945) that incorporation of ^{32}P into DNA is greater in those tissues in which mitotic division is active, e.g. bone marrow and intestinal mucosa, than in resting tissues. But no systematic comparison of incorporation of ^{32}P into DNA, nRNA and cRNA as a function of time has hitherto been made in a series of tissues. Such a study is described in this paper.

EXPERIMENTAL

Young female albino rabbits weighing from 1500 to 1700 g. were used in these experiments. Each animal received 1 mc of ^{32}P as inorganic phosphate by intramuscular injection and 2 hr. later 5 ml. of blood were removed from the ear vein and immediately deproteinized by addition of 0·5 vol. of 30 % (w/v) trichloroacetic acid (TCA). The animals were killed after appropriate time intervals and the tissues immediately excised and chilled in ice. Those not immediately used were stored in solid CO_2 in the frozen state.

For the isolation of nuclei the tissues were disintegrated in a Potter–Elvehjem (1936) homogenizer with plastic pestle in 5 vol. of 0·05M citric acid. The homogenate was strained through nylon gauze and centrifuged at 666 g (International Refrigerated centrifuge) for 10 min. The supernatant fluid was decanted from the nuclear sediment and treated with 0·5 vol. of 30 % (w/v) TCA to precipitate the cytoplasmic protein and nucleic acid.

The nuclear sediment was finely suspended in 0·01M citric acid and centrifuged at 375 g for 5 min. This process

12

was repeated with decreasing speeds of centrifuging until clean nuclei were obtained as seen on microscopic examination (Mirsky & Pollister, 1946). The packed nuclei were then treated with 10 % (w/v) TCA.

The cytoplasmic and nuclear precipitates were washed with 10 % (w/v) TCA (twice), acetone, ethanol, ethanol–chloroform (3:1), ethanol–ether (3:1) (twice), and ether, and dried.

Ribonucleotides were obtained from the dry cytoplasmic powder by extraction with NaCl solution and alkaline hydrolysis of the RNA according to the procedure 2 of Davidson & Smellie (1952b). These nucleotides were then separated by ionophoresis on paper (Davidson & Smellie, 1952a) and were eluted for estimation of P and radioactivity (Davidson & Smellie, 1952a, b). Phosphorus was estimated by the modification of the Allen (1940) method which we have previously employed. Radioactivity was measured in 20th Century Electronics Type M6 liquid counters attached to conventional scaling units.

The dry nuclear powder was incubated overnight at 37° in alkali in the proportions of 1 ml. of 0·5N-KOH to 100 mg. powder. The digest was then acidified with 10N-HClO$_4$ to pH 1, the precipitated DNA and protein spun out and the supernatant fluid brought to pH 3·5 with 5N-KOH. After removal of KClO$_4$, portions of this solution were used for ionophoresis of nucleotides from nRNA as described above for cRNA, and for determination of phosphorus and radioactivity.

The precipitate containing DNA from the nuclear residues was washed with 0·5N-HClO$_4$, ethanol and ether, and dried. DNA was then extracted from this material by application of the method of Kay, Simmons & Dounce (1952). The dry nuclear residue was dispersed in 2·5–10 ml. of 0·9 % (w/v) NaCl and the pH adjusted to 7 with N-NaOH. A 5 % (w/v) solution of sodium dodecyl sulphate (Empicol) in 45 % (v/v) ethanol–water was then added in the proportion of 1 ml./ 10 ml. suspension and the mixture shaken or stirred at intervals over a period of at least 1 hr. NaCl (0·5 g./10 ml. suspension) was added and the mixture centrifuged to remove denatured protein. The supernatant liquid was decanted and 1–2 vol. of ethanol added. The precipitate of the Na salt of DNA was spun down, washed successively with 70 % (v/v) ethanol, ethanol, acetone, and ether and allowed to dry. Portions of the dry powder were taken for the estimation of P and radioactivity.

The sample of blood after precipitation with TCA was centrifuged and the protein-free supernatant fluid treated with Mathison's (1909) reagent (1 ml./10 ml.) and made alkaline with NH$_4$OH (just red to phenolphthalein). The mixture was allowed to stand overnight in the refrigerator and the precipitate of MgNH$_4$PO$_4$ centrifuged down, washed

with dilute NH_4OH and dissolved in dilute HCl. The phosphorus content and radioactivity of this material were then determined. The specific activity of the blood inorganic phosphate was used as a reference standard to which all other specific activity measurements were related.

To obtain the specific activity of the tissue inorganic phosphates, the TCA extracts of the cytoplasmic materials obtained as described above were treated with Mathison's reagent and ammonia as described for blood.

In a few experiments the DNA was degraded to deoxyribonucleotides. About 15 mg. of dry DNA were dissolved in 2 ml. of 0·05 M borate buffer, pH 7·0, containing 16 mg. of $MgSO_4 + 1$ mg. of deoxyribonuclease (McCarty, 1946). The tubes were incubated at 37° for 18 hr., care being taken that no alteration in pH occurred. At the end of this incubation period the tubes were placed in a boiling-water bath for 5 min., cooled and treated with 20 mg. of NaF.

The digest was then further degraded to deoxyribonucleotides with phosphodiesterase prepared from Russell's viper venom obtained from Burroughs Wellcome Ltd. A portion of the purified enzyme prepared as described below was added to the DNA digest and the mixture incubated at 37° for 26 hr. At the end of this period a sample of 0·3 ml. was removed and treated with an equal volume of 1·6 % (w/v) uranyl acetate in 10 % (w/v) TCA. The amounts of inorganic phosphate and of total phosphate soluble in the uranyl acetate reagent were determined and incubation was continued until 90–95 % of the DNA phosphorus had been rendered soluble. With these enzyme preparations the inorganic phosphate liberated amounted to less than 10 % of the total soluble P. When digestion was complete, the tubes were heated in a boiling-water bath for 5 min. to inactivate any phosphatases present. Samples of the digest containing 100–120 μg. P were then applied to strips of Whatmans 3 MM filter paper for ionophoresis. The runs were carried out at pH 4 for 20 hr. when the papers were dried, the nucleotide spots located in ultraviolet light and the material was eluted.

According to the suggestion of Dr E. Volkin, purification of the phosphodiesterase was carried out by fractionation of a solution of Russell's viper venom in 0·05 M-NaCl with icecold acetone (Sinsheimer & Koerner, 1952). The material precipitated by concentrations of acetone up to 50 % (v/v) was discarded and the fractions obtained between 50 and 65 % (v/v) acetone were examined for phosphomonoesterase and phosphodiesterase activity. Very variable results were obtained by this method of fractionation, but in some cases the preparations showed good diesterase activity and negligible contamination with monoesterase. Such fractions were dissolved in water and frozen-dried for subsequent use.

In a few experiments the abdominal viscera and femora of the animals were exposed to X-rays (1000 r) from an Aeromax 12 tube (81 kv, 5 mA), the remainder of the animal being shielded with a sheet of lead. The period of irradiation varied between 10 and 20 min.

In some cases hyperplasia of the bone marrow was induced by subcutaneous administration of 1 ml. of a solution of phenylhydrazine hydrochloride (20 mg./ml.) in 0·9 % NaCl every second day over a period of 10 days.

Red and white blood cell counts were carried out by conventional procedures. Blood films were stained with brilliant cresyl blue for reticulocyte counts and with Leishman's stain for differential counts.

RESULTS

In order to overcome slight variations in the administered dose of ^{32}P and between different rabbits in the series, the results of radioactivity measurements are expressed as relative specific activities where

relative specific activity

$$= \frac{\text{specific activity of fraction (counts/min./100 } \mu\text{g. P)}}{\text{specific activity of blood inorganic phosphate at 2 hr. (counts/min./100 } \mu\text{g. P)}} \times 10\,000.$$

The relative specific activities of the tissue inorganic phosphates at different time intervals after injection of ^{32}P are shown in Fig. 1. Although there is some variation from tissue to tissue, especially at the shorter time intervals, the rate of decline in activity is of the same order in each tissue and it is clear that differences observed in the rates of renewal of the nucleic acids in various tissues cannot be due merely to differences in the rates of penetration of ^{32}P from the blood to the tissues in different organs. We have therefore used the activity of the blood inorganic phosphate as a common standard of comparison for all tissues.

We have previously noted (Davidson & Smellie, 1952 b) that the ribonucleotide fraction obtained from tissues by the method of Schmidt & Thannhauser (1945) is contaminated by other phosphorus compounds to such an extent that its radioactivity does not represent that of the true RNA. Accordingly, in the present studies it was important to establish that the activity of the DNA isolated by the method described truly represented that of the deoxyribonucleotides. The results of two experiments are shown in Table 1 where the relative specific activities of the four deoxyribonucleotides derived from the DNA's of several tissues are compared with those of the whole. In each case the relative specific activity of the DNA is very close to the mean values for the four constituent nucleotides. At the 2 hr. time interval, there is a considerable spread in the activities of the deoxyribonucleotides with deoxyguanylic acid and thymidylic acid generally showing greatest uptake. After 18 hr., however, the values for the four nucleotides are closely similar. We have therefore measured only the activity of whole DNA in subsequent experiments.

Fig. 2 illustrates the variations in relative specific activity with time for DNA, nRNA and cRNA of appendix, bone marrow, small intestinal mucosa, kidney, spleen and thymus. Each point on the curves represents the mean value of several experiments; in all some thirty rabbits were used. The values for DNA are the result of measurements on whole isolated DNA while those for nRNA and cRNA are means of values for the four individual nucleotides obtained by ionophoresis.
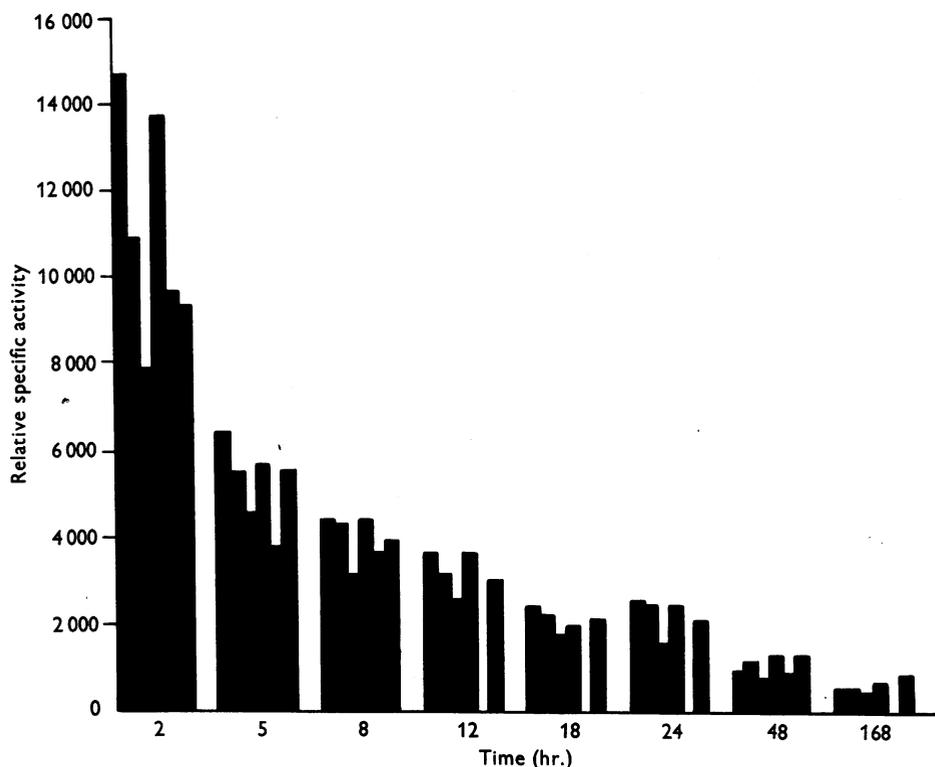
Fig. 1. A series of histograms illustrating the values for the relative specific activities of the inorganic phosphate from several rabbit tissues at various times after administration of 1 mc of ^{32}P. The columns in each group represent from left to right, appendix, bone marrow, small intestinal mucosa, kidney, spleen and thymus. In a few cases the amount of inorganic phosphorus obtained from spleen was too small to permit accurate estimation; in these instances the column for spleen has been left blank.

Table 1. *Comparison of the relative specific activities of whole DNA and of individual deoxyribonucleotides from different rabbit tissues*

Animals were killed 2 and 18 hr. after administration of isotope.

Tissue	Deoxyadenylic acid	Deoxyguanylic acid	Deoxycytidylic acid	Thymidylic acid	Mean	Whole DNA
2 hr.						
Appendix	1030	1220	1130	1970	1340	1373
Bone marrow	1155	1460	1165	1480	1315	1290
Intestinal mucosa	233	326	218	326	276	258
Liver	59	65	45	48	54	61
Thymus	335	425	342	546	412	434
18 hr.						
Appendix	2480	2525	2515	2700	2555	2692
Bone marrow	2830	—	3470	2885	3062	2761
Intestinal mucosa	572	658	618	472	555	585
Thymus	1100	1050	1335	1025	1128	1116

The time/activity curves for DNA make it possible to classify the tissues into three main groups: (a) Kidney in which the incorporation of ^{32}P into DNA is negligible. The curve for cRNA shows a moderate rise while that for nRNA gives a very definite peak within the first 10 hr. (b)

Intestinal mucosa, spleen and thymus in which th DNA shows an appreciable incorporation of ^{32}P but the curve shows no sharp maximum and remains generally below that for cRNA. (c) Appendix and bone marrow in which the activity of the DNA rises very sharply indeed to a high maximum at

12-2

8–10 hr. The curve for cRNA also rises very sharply and reaches its maximum at about the same time but for the first 20 hr. or so the activity of cRNA is less than that of DNA while that of nRNA remains consistently higher. Indeed in all the tissues studied the relative specific activity of nRNA rises more rapidly and to a higher level than does that of either DNA or cRNA. The nRNA's of appendix and bone marrow probably reach peak activity within 2 hr. of the administration of ^{32}P, while in the other

four tissues the maximum occurs between 7 and 10 hr.

The curves for cRNA fall into two groups, appendix and bone marrow, where the maximum approaches that of the DNA at 10–12 hr., and the remaining tissues in which the cRNA rises more slowly to a very broad maximum.

A general comparison of all the curves shows that by 48 hr. the DNA, nRNA and cRNA of most of the tissues have reached a common level which main-
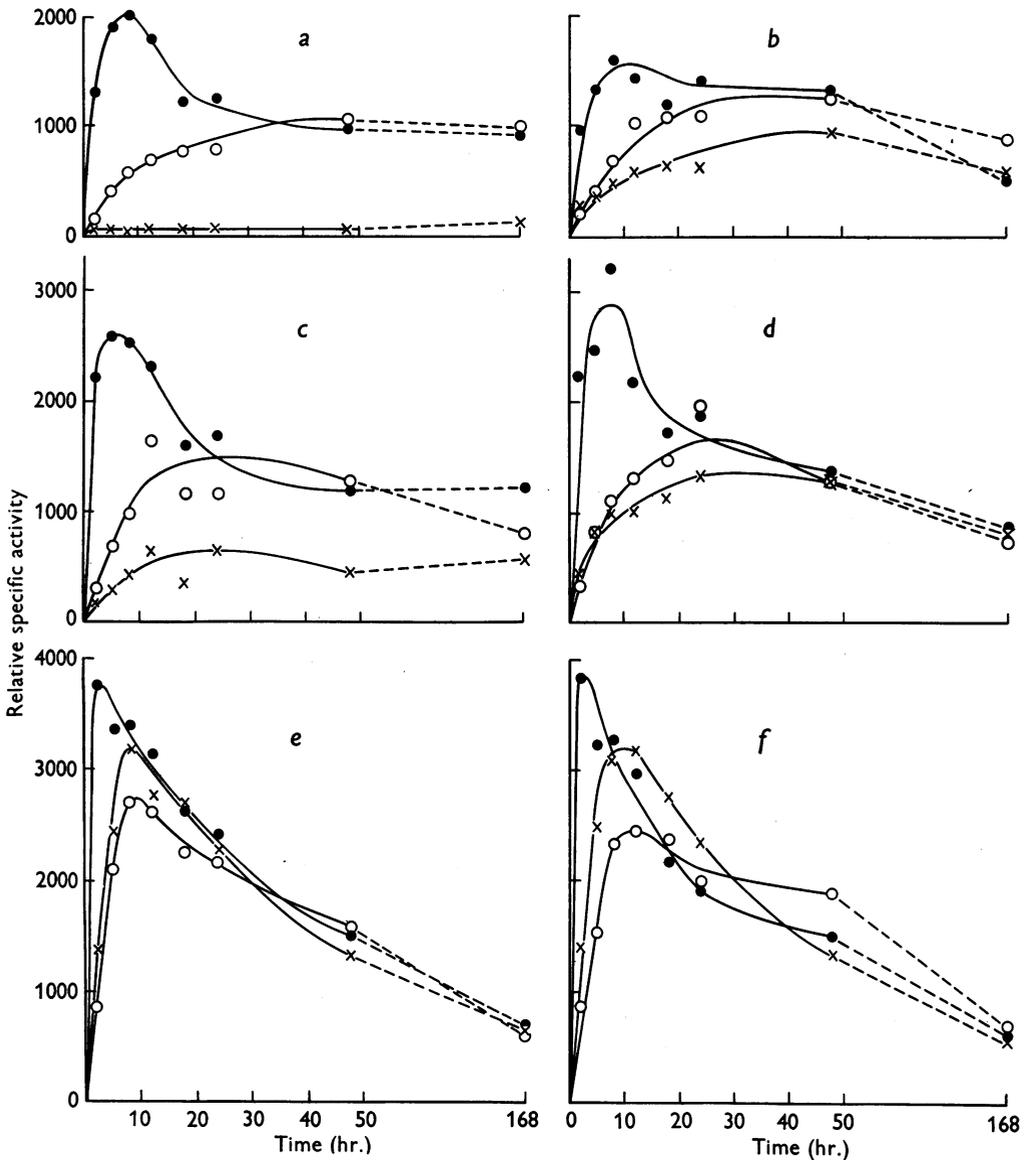

Fig. 2. The relative specific activities of the DNA's (×), nRNA's (●) and cRNA's (○) of different rabbit tissues at intervals between 2 hr. to 7 days after the intramuscular injection of 1 mc ^{32}P. a, kidney; b, small intestinal mucosa; c, spleen; d, thymus; e, appendix; f, bone marrow.

tained up to at least 7 days. The exception to this general observation are kidney and spleen DNA.

These curves provide a pattern for the incorporation of ^{32}P into the nucleic acids of normal rabbits.

In Table 2 the results of four experiments are illustrated in which the rabbit viscera and femora were exposed to X-irradiation before the administration of the isotope. It is clear that at short time intervals after irradiation there is little effect upon the blood erythrocyte population or on the total white cell counts. However, there is a rapid increase in the proportion of granulocytes and a corresponding decrease in non-granulocytes. At longer time intervals there is a decrease in both erythrocytes and total leucocytes. It should be noted that normal rabbit blood contains approximately equal numbers of granulocytes and non-granulocytes (Scarborough, 1931).

appendix and bone marrow are renewed at diminished rates after irradiation, the maximum effect being found after 20 hr. The DNA in these tissues, however, seems to be affected to a greater degree than are either nRNA or cRNA. Thymus nRNA and cRNA again are little different from normal 2 hr. after exposure to X-rays but again there is a delayed effect which appears after 20 hr. and is still apparent at 49 hr. Shortly after exposure, kidney nRNA and cRNA seem to exhibit slightly elevated values but later show a decline in activity.

The effect of administration of phenylhydrazine is to produce a haemolytic anaemia which results in hyperplasia of the bone marrow and a very pronounced enlargement of the spleen. The severity of the anaemia can be judged by the proportion of reticulocytes in the peripheral blood. The uptake of ^{32}P by the nucleic acids of different organs in these circumstances is illustrated in Table 3 which shows

Table 2. *The effect of X-irradiation* (1000 r) *of abdominal viscera and femora on the incorporation of* ^{32}P *into the DNA, nRNA and cRNA of rabbit tissues*

Each animal received 1 mc of ^{32}P as inorganic phosphate 2 hr. before being killed.

Expt. no.	Time between irradiation and killing (hr.)	Erythrocytes (% of pre-irradiation value)	Leucocytes (% of pre-irradiation values)			Tissue	Relative specific activity		
			Total	Granulocytes	Non-granulocytes		DNA (% of normal)	nRNA (% of normal)	cRNA (% of normal)
32	2	100	91	275	24	Appendix	41	62	75
						Bone marrow	56	56	50
						Kidney	77	146.	154
						Thymus	119	110	112
33	2	100	103	213	56	Appendix	27	57	65
						Bone marrow	71	99	68
						Kidney	80	134	120
						Thymus	102	78	91
37	20	103	54	133	10	Appendix	17	31	32
						Bone marrow	23	24	17
						Kidney	92	75	87
						Thymus	37	32	46
39	49	86·5	68	276	28	Appendix	33	52	53
						Bone marrow	76	55	58
						Kidney	120	67	59
						Thymus	46	52	64

The effect of irradiation on ^{32}P incorporation is most marked in the DNA of appendix and bone marrow in which the relative specific activities fall to about 35 and 60 % of the respective normal values after 2 hr. By 20 hr. an even more marked drop in uptake of ^{32}P by the DNA of appendix and bone marrow is apparent, while by 49 hr. these tissues show signs of recovery with respect to DNA turnover. The renewal of thymus DNA is not apparently affected within 2 hr. of irradiation, but by 20 and 49 hr. there is a marked decline in ^{32}P uptake. Renewal of kidney DNA appears to fall slightly after irradiation but the normal level of uptake in kidney DNA is so small (Fig. 2a) that these changes are of doubtful significance. Both nRNA and cRNA of

Table 3. *Effect of hyperplasia of rabbit bone marrow caused by haemolytic anaemia induced by phenylhydrazine on the uptake of* ^{32}P *by the DNA, nRNA and cRNA of different tissues*

1 mc of ^{32}P was administered as inorganic phosphate 2 hr. before killing. Red cell count was 58 % of normal. Reticulocyte count was 85 % of total red cells. All figures are given as percentages of the normal values and represent the means of two experiments.

Tissue	DNA	nRNA	cRNA
Appendix	82	89	103
Bone marrow	245	116	192
Kidney	95	115	100
Spleen	980	131	242
Thymus	99	85	107

that the relative specific activity of bone marrow DNA is increased by a factor of 2–3, while the spleen DNA activity is nearly 10 times the normal value. Appendix, kidney and thymus DNA remain unchanged.

The uptake of ^{32}P by the nRNA of all five tissues was not so greatly affected by the phenylhydrazine treatment, the most marked effect being on the spleen which showed a 30% increase in activity. The activity of cRNA of bone marrow and spleen was about twice that in normal animals while that of appendix, kidney and thymus was apparently unchanged.

DISCUSSION

In this work the convention of expressing the results of radioactivity measurements in relation to the blood inorganic phosphate at 2 hr. has been followed. This method of expression tends to eliminate the effects of slight variations in dosage and in absorption from the site of injection from one animal to another. It also tends to minimize the effects of slight differences in weight between animals.

One of the major difficulties in this type of work is to find a suitable factor in terms of which the results of radioactivity measurements may be expressed. Tissue inorganic phosphate may for instance be used, but the necessary figures can be obtained only at the time of killing and decline almost logarithmically throughout the experiment (Fig. 1). Another common method is to relate specific activity determinations to the administered dose of isotope. While this has certain advantages, it takes no account of variations in absorption from the site of injection. We feel that the method that we have adopted is on the whole the most satisfactory available.

In view of the ease with which nucleic acid fractions can be contaminated by phosphorus compounds of higher specific activity (Davidson & Smellie, 1952b) we have thought it advisable to show that radioactivity measurements on isolated DNA's truly represent the activity of the phosphorus in the deoxyribonucleotides. This has been done by degrading the DNA to the mononucleotides by means of deoxyribonuclease and phosphodiesterase (Little & Butler, 1951; Hurst, Little & Butler, 1951). The hydrolysis of the deoxyribonuclease digest with phosphodiesterase proved to be the most difficult stage, since the enzyme preparations could not be consistently freed from monoesterase activity. The chromatographic method of Hurst & Butler (1951) in our hands did not yield satisfactory diesterase preparations but acetone fractionation along the lines of the procedure of Sinsheimer & Koerner (1952) provided some suitable fractions. The ionophoretic method for separating ribonucleotides (Davidson & Smellie, 1952a) was easily modified to enable the deoxyribonucleotides to be resolved and proved convenient in these experiments.

The results of experiments in which the radioactivity of isolated DNA is compared with that of the corresponding nucleotides (Table 1) make it clear that the routine separation of the nucleotides is unnecessary and that it is justifiable to take the radioactivity of the isolated DNA as a measure of the ^{32}P content of the DNA.

In calculating the mean value for the relative specific activity of the four deoxyribonucleotides in DNA for comparison with that of whole DNA and, in taking the mean value of the relative specific activities of the four ribonucleotides as representative of whole RNA, no allowance has been made for the fact that in neither type of nucleic acid are the constituent nucleotides present in equimolecular proportions. In our opinion this would give rise to a serious error only if the activity were particularly high or low in any one nucleotide which was present in exceptionally high or low proportions and this is not the case.

Many workers have studied the incorporation of isotopes into the DNA of different tissues, but few systematic comparisons of different tissues at various time intervals have been made. Hevesy & Ottesen (1943) determined the daily percentage renewal of DNA in different rat tissues and found it to decrease in the order: small intestinal mucosa, spleen, testes, muscle, liver, kidney and brain. In a later paper Hammarsten & Hevesy (1946) studied the renewal of RNA and DNA in rat liver, spleen and intestine. Andreasen & Ottesen (1945) examined the replacement of P in the DNA of various lymphoid tissues of young, adult and old rats. In general, uptake of isotope by the DNA of lymphoid tissues was high, decreasing in the order: bone marrow, thymus, intestinal lymph nodes, skin lymph nodes and spleen.

In no case, however, has a systematic comparison of the time/activity curves of DNA, nRNA and cRNA been made. It is felt that such a study is of considerable importance in establishing the normal pattern of P incorporation into the nucleic acids of different tissues and also as a contribution towards the question of the relationship of DNA turnover to the mitotic activity of a tissue.

The outstanding feature of these experiments is that the tissues may be divided into three main categories according to the turnover of their DNA. In kidney, where mitotic activity is negligible, the DNA turnover is very low indeed and the time/activity curve is comparable with that found for liver (Smellie et al. 1953). In tissues of moderate mitotic activity such as intestinal mucosa, spleen and thymus, renewal of DNA is fairly rapid, while in

bone marrow where cell division is brisk, the DNA activity is very high indeed. The pattern of activity of appendix is surprisingly like that of bone marrow with the turnover of DNA much more rapid than is found in the other lymphoid organs. Although the appendix was carefully washed after excision it could obviously not be completely freed from the bacterial flora which abounds in its crypts and even in the submucosa (Digby & Enticknap, 1954), but it seems to us unlikely that such bacteria could be present in sufficient amount to influence the figures for nucleic acid turnover to any appreciable extent.

While our results in general indicate a correlation between the mitotic activity of a tissue and the renewal of its DNA phosphorus, we have not made any direct quantitative measurements of mitotic activity. Such measurements have, however, been made by Stevens, Daoust & Leblond (1953) and Daoust, Bertalanffy & Leblond (1954), who studied the renewal of DNA phosphorus in relation to mitosis in rat liver, intestine and lung and concluded that the DNA of both daughter cells resulting from mitosis is newly formed. This of course implies that there is a direct relationship between DNA turnover and mitosis.

It has been observed by many authors (for literature references see Smellie et al. 1953) that nRNA shows a much more rapid turnover than does cRNA and this is amply brought out in the present experiments. In appendix and bone marrow the curve for nRNA rises rapidly to a high value, and almost certainly reaches a maximum well within 2 hr. The remarkably rapid rise in activity with time in all six tissues emphasizes the important but still not clear metabolic role for nRNA.

Previous studies on the nRNA's of rabbit-liver cytoplasmic fractions (Smellie et al. 1953) have shown little difference in the rate of renewal of mitochondrial and microsomal RNA, although the RNA of the cell sap is metabolically more active than either of these. Similar results have been recorded by Barnum & Huseby (1950) for normal mouse liver, and Barnum, Huseby & Vermund (1953) for mouse mammary carcinoma. In the present series of experiments we have considered cytoplasmic RNA as a single metabolic entity, since the disintegration of the tissue was carried out in citric acid in which separation of the morphological components of the cytoplasm is not possible, but our previous experience with liver, has shown that the RNA's of the cytoplasmic components give curves which are closely related and quite distinct from the curve for nRNA (Smellie et al. 1953). In the present series of experiments the patterns of ^{32}P incorporation into cRNA's are similar in intestinal mucosa, kidney, spleen and thymus but in appendix and bone marrow the curves rise much more steeply and exhibit more sharply defined

maxima. Although cytoplasmic material is moderately abundant in bone marrow, it is obtainable only in small amounts from appendix, as might be expected from the histological picture. Nevertheless, the separate characteristic features of the curves for cRNA and nRNA in appendix confirm that differences in morphological origin are accompanied by differences in metabolic activity.

It should also be noted that the curves for the three nucleic acids bear quite different relationships to each other in the different tissues. This would indicate that the metabolic interrelationship between the nucleic acids may show wide variations from one tissue to another and emphasizes the danger of drawing general conclusions regarding mammalian cells from any one tissue. For instance, the role of nRNA as a possible precursor of cRNA may be much more improbable in one tissue than in another.

An interesting feature of the curves is the retention of ^{32}P after 7 days in all three forms of nucleic acid, although this can be explained in part by reutilization. There is evidence in certain tissues such as kidney of very little fall during the last 5 days.

The effect of X-irradiation of viscera and femora on the uptake of ^{32}P by the nucleic acids of appendix, bone marrow, kidney and thymus is of considerable importance. In agreement with the observations of Hevesy (1945, 1949, 1951), Kelly & Jones (1950), Abrams (1951), Vermund, Barnum, Huseby & Stenstrom (1953), Harrington & Lavik (1953) and Payne, Kelly & Entenman (1952), it has been shown that a marked inhibition of ^{32}P uptake by the DNA of the irradiated tissues occurs within 2 hr. of treatment. It has also been observed that at this short interval following exposure there is little effect upon the uptake of isotope by the DNA of thymus which was not irradiated and probably no effect on the DNA of kidney which was. Even at 2 hr., however, there is evidence of a considerable disturbance in the production of blood granulocytes and non-granulocytes. These changes are even more marked by 20 hr. after irradiation, but by 49 hr. the pattern appears to be returning to normal. It is important to note that at these later times the uptake of tracer by thymus DNA is considerably reduced, although kidney DNA remains more or less normal.

The delay in the reduction of thymus DNA renewal is of interest since this might represent an indirect effect of the irradiation for which two explanations may be put forward. First, in accord with the suggestions of Hevesy (1945, 1951), Holmes (1947, 1949), Holmes & Mee (1952) and Kelly & Jones (1950), it may be that some humoral agent is produced as a result of irradiation which affects non-irradiated tissues. The second possibility is

that cellular material produced in the appendix is subsequently taken up by the thymus.

The observation that kidney DNA turnover is little affected by irradiation provides further confirmation that tissues in which cell division is minimal are much less sensitive to X-rays than are actively proliferating organs. This in turn confirms the suggested relationship between cell division and uptake of ^{32}P by DNA.

In bone marrow, appendix and thymus the effect of irradiation is less marked upon the uptake of the isotope by the nRNA and cRNA than on the DNA. In kidney the renewal of nRNA and cRNA appears to be slightly increased initially, although it is depressed at the longer time intervals.

Conflicting reports appear in the literature regarding the effects of X-irradiation on RNA metabolism. Thus, Abrams (1951) found that X-irradiation of intestine and bone marrow markedly reduced the rate of synthesis of both RNA and DNA. Holmes (1947, 1949), using tumour tissue, also found RNA turnover to be inhibited by X-rays but to a lesser extent than DNA synthesis. These authors did not distinguish between nRNA and cRNA but the general pattern of inhibition of RNA turnover as well as of DNA turnover is confirmed by our experiments with appendix, bone marrow and thymus and is in contrast to the findings of Vermund et al. (1953), who found no effect of X-rays on the turnover of RNA in mouse carcinoma although the DNA was markedly affected. The only tissue in which we found an increase in the turnover of RNA as the result of irradiation was kidney, in which mitotic activity is very low and it is of interest to note that Payne et al. (1952) found a similar increase in the cRNA of the livers of irradiated rats. Klein & Forssberg (1954) have observed that X-irradiation of the Ehrlich mouse ascites tumour causes a fall of about 30 % in the uptake of (2-^{14}C) glycine by the tumour DNA and whole RNA. These findings are at variance with previous estimates of inhibition of nucleic acid synthesis by X-irradiation based on purely chemical analyses (Klein & Forssberg, 1954), which suggested that DNA synthesis fell to about 33 % of the control level while RNA synthesis was unimpaired.

In the experiments with animals with hyperplastic marrow the metabolism of the nucleic acids of the five tissues examined is changed only in the bone marrow and spleen. The greatest differences are evident in the DNA of bone marrow and spleen and in cRNA. In spleen the figure for nRNA is also higher than normal but incorporation into bone marrow nRNA is only slightly increased. These preliminary findings again suggest a relationship between cell division and DNA turnover since the mitotic activity of bone marrow is greatly increased in the anaemic animals. The considerable enlarge-

ment noted in the spleens of these animals also indicates increased cell division. The results might suggest a system for protein and DNA synthesis either independent of nRNA or dependent more on cRNA than on nRNA, but further experiments would be essential before such a conclusion could be drawn since it is possible that there has been a complete alteration in the relative time/activity curves for nRNA, cRNA and DNA in these hyperplastic tissues.

SUMMARY

1. A comparison has been made between the radioactivity of the whole deoxyribonucleic acids (DNA's) isolated from a number of different rabbit tissues after ^{32}P administration and of their constituent deoxyribonucleotides. The conclusion is drawn that it is justifiable to take the activity of the isolated DNA as a true measure of phosphorus uptake.

2. The course of incorporation of ^{32}P into the DNA, nuclear ribonucleic acid (RNA), cytoplasmic RNA and inorganic phosphate of rabbit appendix, bone marrow, intestinal mucosa, kidney, spleen and thymus has been followed between 2 hr. and 7 days. The tissues may be divided into three groups with respect to DNA turnover: (a) kidney in which the renewal of DNA is very small; (b) intestinal mucosa, spleen and thymus which show moderate turnover rates for DNA; (c) appendix and bone marrow in which the rate of DNA renewal is very rapid.

3. In all tissues the turnover of nuclear RNA was much more rapid than that of DNA or cytoplasmic RNA. Only in bone marrow and appendix did cytoplasmic RNA give lower specific activity values than DNA.

4. X-irradiation of abdominal viscera and femora produced a marked inhibition of ^{32}P incorporation into DNA, nuclear RNA and cytoplasmic RNA of appendix and bone marrow. A delayed depression of the uptake of ^{32}P by the DNA, nuclear RNA and cytoplasmic RNA of thymus was also observed. Only small changes were noted in the kidney nucleic acids, both nuclear RNA and cytoplasmic RNA showing slightly elevated activities 2 hr. after irradiation and low values after 20 and 49 hr.

5. Phenylhydrazine induced haemolytic anaemia with resultant hyperplasia of the bone marrow causes a twofold increase in the activity of bone marrow DNA and a tenfold increase in the activity of spleen DNA. The cytoplasmic RNA's of bone marrow and spleen also showed greatly increased activities but comparatively small changes were found in the uptake of ^{32}P by the nuclear RNA's of these tissues. The metabolism of appendix, kidney and thymus nucleic acids did not appear to be affected by this treatment.

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A Chemical Method for the Determination of Oestriol, Oestrone and Oestradiol in Human Urine

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In connexion with a research programme to study the clinical significance of variations in the urinary excretion and metabolism of the oestrogens, an accurate method, suitable for day-to-day routine use, was required for the separate determination of oestriol, oestrone and oestradiol-17β in the low concentrations found in the urine of men and non-pregnant women. Since all available methods lacked the desired accuracy, sensitivity, specificity and convenience, it was decided to attempt to develop a more suitable procedure. The project was organized jointly with the Department of Biochemistry, and papers dealing with one aspect of the problem (Brown, 1952; Bauld, 1954) have already been published. The present paper describes in detail one complete procedure which appears to be more satisfactory in many respects than any that have hitherto been described, and has been used on a routine basis in clinical investigations for the past 18 months. A preliminary communication in which the method was briefly described has already been presented (Brown, 1955).

The method was evolved from the one described by Clayton (1949) and incorporates a new phase-change purification step involving methylation of the phenolic fraction. The methylated oestrogens are then further purified and separated from one another by chromatography on alumina columns and are estimated colorimetrically using an improved modification of the Kober colour reaction