4. The properties of the flavan-4-ol in comparison with those of the flavan-3-ol suggest that the hydroxyl group in position 4 might be involved in the biochemical condensation of flavan-3:4-diols, and also in the redness developed in these compounds and in associated or related condensed tannins.

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Diphosphopyridine Nucleotide Pyrophosphorylase in the Nuclei Isolated from Poisoned and Regenerating Rat Liver

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The stimulus for this work was a paper by Morton (1958) in which it was suggested that, since diphosphopyridine nucleotide is required for more enzymic reactions than any other known coenzyme, alteration of its rate of synthesis could modify cell division and differentiation and perhaps lead to malignant growth. An important enzyme in its synthesis is diphosphopyridine nucleotide pyrophosphorylase, which is located exclusively in the nuclei (Hogeboom & Schneider, 1952). Low activities of this enzyme were obtained in the nuclei from spontaneous mouse-mammary-gland carcinoma, hepatomas after feeding aminoazobenzene, Ehrlich ascites cells, post-operative human tumours and foetal mouse liver (Morton, 1958; Branster & Morton, 1956). These findings were correlated with

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the low diphosphopyridine nucleotide content of these tissues, and it was suggested that in rapidly growing cells synthesis of new cell material occurs against a limiting supply of diphosphopyridine nucleotide from the nucleus. When the diphosphopyridine nucleotide falls to a critically low concentration, the cells divide, thus providing more nuclear enzyme in relation to the cytoplasm.

It therefore seemed interesting to examine the diphosphopyridine nucleotide pyrophosphorylase of the nuclei from livers from animals after various poisons causing cell necrosis and also from livers regenerating after partial hepatectomy. Before this work was attempted an examination was made of isolation procedures for nuclei. Some modifications were made and some properties of nuclei obtained were compared with those obtained in other laboratories.

EXPERIMENTAL

Abbreviations. The exchange of ^{32}P (added as inorganic phosphate) with the phosphorus atoms of ATP, ^{32}P–ATP exchange reaction; deoxyribonucleic acid phosphorus, DNA P.

Materials

Animals. Female rats (150–200 g.) were used throughout. Sprague–Dawley rats were used for the experiments where DPN synthesis was measured and albino rats of the Porton strain for all other experiments. The animals were allowed free access to food at all times.

Special chemicals. The following chemicals were obtained from the sources indicated: disodium salt of ATP, nicotinamide mononucleotide, alcohol dehydrogenase (Sigma Chemical Co., St Louis, Mo., U.S.A.); nicotinamide and ethionine (Roche Products). Beryllium sulphate, dimethylnitrosamine, CCl_4 and ethionine were used as hepatotoxic agents. Partial hepatectomy was performed by the procedure of Higgins & Anderson (1931).

Methods

Isolation of nuclei. The nuclei were isolated by a modification of the basic technique of Hogeboom, Schneider & Striebich (1952). Schneider & Petermann (1950) showed that nuclei which were isolated in 0·25 M-sucrose agglutinate, but that it is possible to prevent this by adding small amounts of bivalent ions. They used 1·8 mM-$CaCl_2$ in 0·25 M-sucrose and calcium has been used in most procedures (Hogeboom *et al.* 1952; Allfrey, Mirsky & Osawa, 1957; Creasey & Stocken, 1959). It was, however, shown in the original work of Schneider & Petermann that magnesium could replace calcium. We have preferred to use magnesium as a more usual intracellular ion. The concentrations of magnesium used were empirically determined and were the maximum that could be used without causing gross agglutination of the mitochondria, thus preventing major contamination of the nuclei with mitochondria.

After decapitation of the rats, the livers were perfused *in situ* through the inferior vena cava with about 30 ml. of ice-cold 0·9% NaCl solution followed by 60–80 ml. of ice-cold 0·25 M-sucrose containing 0·4 mM-$MgCl_2$. The lobes were manipulated during perfusion in order to remove blood. In some of the poisoned rats (after dimethylnitrosamine and to a less extent after ethionine) it was impossible to obtain completely blood-free livers. The livers were removed, roughly dried, weighed and homogenized in 30 ml. of 0·25 M-sucrose containing 3 mM-$MgCl_2$, in a Potter–Elvehjem-type homogenizer with a smooth glass tube and a Perspex pestle (Aldridge, Emery & Street, 1960), with a gap of 0·01 in. (difference in diameter, 0·02 in.) and a speed of 1900 rev./min. Homogenization was complete after 15 up-and-down movements. The resulting homogenate was diluted to a volume (ml.) equal to 10 times the weight of the liver (g.) with 0·25 M-sucrose containing 3 mM-$MgCl_2$ and then filtered through one layer of flannelette (W.F. 12, J. H. Bounds Ltd., Manchester) to remove connective tissue and unbroken cells. A portion (10 ml.) of the filtered homogenate was layered over 20 ml. of 0·34 M-sucrose containing 0·4 mM-$MgCl_2$ and centrifuged for 10 min. at 700g at 0° in a swing-out head. The supernatant was pipetted off the sedimented material, which was resuspended in 5 ml. of 0·25 M-sucrose containing 0·4 mM-$MgCl_2$. Sucrose (10 ml. of 0·34 M) containing 0·4 mM-$MgCl_2$ was layered under this suspension and was centrifuged as before. The supernatant was removed and the procedure repeated. The final sediment was resuspended in 1 ml. of 0·25 M-sucrose containing 0·4 mM-$MgCl_2$/10 ml. of 10% (w/v) homogenate. All manipulations were carried out as near to 0° as possible.

Succinic dehydrogenase. This was determined by a modification (Aldridge & Johnson, 1959) of the manometric method of Quastel & Wheatley (1938). Dilution of the fraction with water before assay was essential to obtain maximum activity.

Adenosine triphosphatase. This was determined by the procedure described by Aldridge & Stoner (1960).

Protein. This was determined by the modified (Aldridge, 1957) biuret method of Robinson & Hogden (1940) and the results are expressed as albumin.

Deoxyribonucleic acid. This was determined by the diphenylamine method of Dische (1930), modified by Burton (1956).

Diphosphopyridine nucleotide pyrophosphorylase. This was determined by a modification (Branster & Morton, 1956) of the original method of Kornberg (1950). The medium contained, in 0·8 ml., 0·3 ml. of 0·25 M-glycylglycine buffer brought to pH 7·4 with KOH, 5 μmoles of ATP, 3 μmoles of nicotinamide mononucleotide, 300 μmoles of nicotinamide, 15 μmoles of $MgCl_2$ and 0·2 ml. of a suitable suspension of nuclei. The mixture was shaken for 20 min. at 38° in air and the reaction was stopped by the addition of 0·8 ml. of N-$HClO_4$. Blanks were made either at zero time by adding the nuclei after the $HClO_4$ or by omitting the nicotinamide mononucleotide from the reaction mixture. No difference was found between the two blanks and low values for DPN content were always obtained. After chilling in ice, the tubes were centrifuged at 0°. The sediment was resuspended in N-$HClO_4$ and recentrifuged. The supernatants were combined, brought to approx. pH 8 by adding NaOH, with phenol red as an internal indicator, and diluted to 2·5 ml.; 1 ml. was used for the determination of DPN by using alcohol dehydrogenase as described by Kornberg (1950). DPN formation in 20 min. was linearly related to the amount of nuclei used and with a fixed amount of nuclei was linear with time.

RESULTS

Properties of the nuclear fraction

Contamination of the nuclear fraction. There are two major difficulties in obtaining pure suspensions of nuclei. One is contamination with whole liver cells and erythrocytes and the other is contamination by mitochondria. In our experience the conditions of homogenization to produce complete disruption of all liver cells also causes extensive damage to the nuclei. The minimum clearance and maximum speed of rotation of the pestle has therefore been chosen to produce minimal damage to the nuclei as judged by their microscopical appearance. Erythrocytes have been removed by perfusion of the liver and the major amount of intact whole cells has been filtered off with flannelette. As discussed later this is not entirely satisfactory for some purposes, but preliminary experiments with a fine nylon bolting cloth have been promising. The bivalent ions used to preserve the nuclear membrane can cause agglutination of mitochondria. Contamination of nuclei with mitochondria (other than those in whole cells) is caused by such agglutination and subsequent sedimentation with the nuclei. The maximum concentration of magnesium chloride that will cause minimal sedimentation of the mitochondria with the nuclei has been used in the various solutions. The contamination by whole liver cells and mitochondria has been determined by measurements of succinic dehydrogenase. The final procedure adopted gives suspensions of nuclei containing 1–1·7 % of the succinic dehydrogenase of the original homogenate (Table 1). Determinations of the ^{32}P–ATP exchange reaction in the suspension of nuclei gave an activity that could be accounted for by a contamination with less than 2 % of the activity of the mitochondria in the original homogenate. In common with the ^{32}P–ATP exchange in mitochondria (Boyer, Luchsinger & Falcone, 1956; Löw, Siekevitz, Ernster & Lindberg, 1958), the ^{32}P–ATP exchange mediated by the suspension of nuclei was inhibited by 2:4-dinitrophenol. The simplest explanation of these findings is that there is a slight contamination by mitochondria or whole cells, equivalent to 1–2 % of the original homogenate, and that the nuclei do not contain a system of oxidative phosphorylation comparable with that in mitochondria.

Nuclear size. The preparations of nuclei were examined microscopically either as fresh suspensions by phase contrast or as smears stained by the Feulgen technique. Both methods showed most of the nuclei well shaped, round, with one or more

Table 1. *Succinic dehydrogenase, the* [^{32}P]*phosphate–adenosine triphosphate exchange reaction and adenosine triphosphatase in nuclear fractions from rat liver*

The adenosine triphosphatase measurements were determined on the same preparation as in Expt. 1 under succinic dehydrogenase.

Succinic dehydrogenase

Expt. no.	Homogenate	Nuclear fraction	Percentage in nuclear fraction
	(μl. of CO_2/min./g. wet wt. of liver)		
1	435	7·4	1·7
2	284	3·4	1·2

Adenosine triphosphatase

Additions	Homogenate	Nuclear fraction	Percentage in nuclear fraction
	(μg.atoms of P/hr./g. wet wt. of liver)		
Nil	573	47·5	8·3
	(μg.atoms of P/hr./mg. of protein)		
Nil	3·22	6·55	—
33 μM-2:4-Dinitrophenol	5·93	7·45	—

^{32}P–ATP *exchange reaction*

Additions	Mitochondrial fraction	Nuclear fraction	Percentage in nuclear fraction
	(counts/min./μg.atom of exchangeable P/g. wet wt. of liver)		
Nil	20 100*	353	1·7
33 μM-2:4-Dinitrophenol	—	65	—

* Taken from Aldridge & Threlfall (1961).

visible nucleoli. No cytoplasmic tags were adherent to the nuclei. The preparations were contaminated by a few unbroken cells, nuclear fragments and a few mitochondria and very occasionally red cells.

Experiments were carried out to determine the effect of varying the concentration of sucrose in the suspending medium upon nuclear diameter. The results are given in Fig. 1 and show that nuclear diameter varies inversely with the concentration of sucrose. The value for nuclear diameter in 0·25 M-sucrose is 8·1 μ which agrees with the values of 8·26 μ and 7·5–8·5 μ given for normal nuclei measured in fixed and stained material by Abercrombie & Harkness (1951) and Rather (1958) respectively. These results indicate that a concentration of sucrose of about 0·25 M is suitable for the isolation of nuclei, though the accuracy of this procedure is not such that it is possible to say that 0·25 M is better than 0·3 M.

Formation of 'nuclear gels'. Damage to mitochondria releases an enzyme that has the property of preventing the formation of gels when the nuclei are subsequently treated with alkali at pH 9–10 (Dounce, Witter, Monty, Pate & Cottone, 1955; Dounce & Monty, 1955). These workers consider that DNA is normally bound firmly to some protein component by non-ionic linkages and that it is only by exposure to damaged mitochondria that these linkages are broken. On this view the formation of gels by treatment with alkali may be considered a property of 'normal' nuclei. The preparation in this paper formed very quickly a transparent viscous gel when the pH was brought to 9–10.

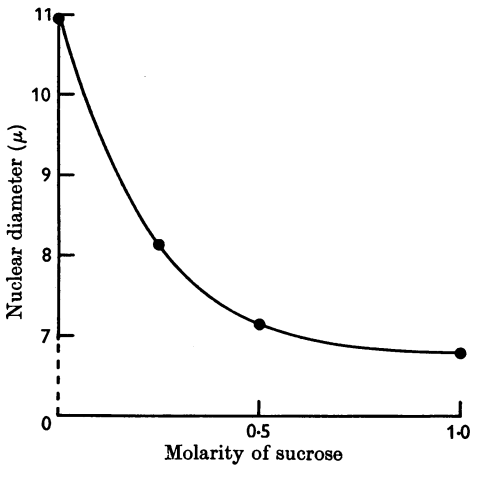

Fig. 1. Mean diameter of rat-liver nuclei suspended in sucrose of various molarities. The nuclei were suspended in the sucrose solutions for 1 hr. at 0° before measurement of their diameter with a phase-contrast microscope with a calibrated eyepiece. At each concentration of sucrose 60–80 measurements were made.

Adenosine triphosphatase in nuclear fractions. Although several workers have measured adenosine triphosphatase activity in nuclear fractions, there are difficulties in the interpretation of the results. By both the non-aqueous method of isolation (Behrens, 1939; Allfrey, Stern, Mirsky & Saetren, 1952) and that with aqueous solutions (Lang & Siebert, 1951; Philpot & Stanier, 1956; Chauveau, 1952) preparations from liver and kidney are obtained which contain adenosine triphosphatase activity (Stern, Allfrey, Mirsky & Saetren, 1952; Novikoff, Hecht, Podber & Ryan, 1952; Lang & Siebert, 1951; Fischer, Siebert & Adloff, 1959; Miller & Goldfeder, 1960). In most cases there is much doubt either about the degree of contamination by other constituents of the cytoplasm or about the effects of the solvents used in the non-aqueous method upon such enzyme(s). Nuclei prepared by the method described in this paper have appreciable adenosine triphosphatase activity (Table 1), the specific activity of the nuclei being higher than that of the homogenate. Succinic-dehydrogenase determinations indicate a contamination of the nuclear fraction by 1·7 % of the mitochondria of the homogenate. In contrast the nuclear fraction contains over 8 % of the adenosine triphosphatase. The addition of 2:4-dinitrophenol stimulated the activity of the homogenate by 85 %, but the nuclear fraction by only 8 %. The slight stimulation of the nuclear fraction is not more than 1·4 % of the stimulation obtained in the homogenate and is presumably due to the mitochondria. It is clear therefore that our nuclear fraction contains an adenosine triphosphatase not associated with mitochondrial contamination, and therefore rat-liver nuclei contain an adenosine triphosphatase.

Yield of nuclei. The efficiency of the recovery of nuclei from the homogenate by centrifuging is best judged by determinations of DNA. The DNA recovered in three experiments was 66, 55 and 70 % of that present in the original unfiltered homogenate. We have been unable satisfactorily to determine DNA in the filtered homogenate. As far as we can deduce from our experiments, this appears to be due to the elution from the flannelette of some substance that interferes in the method of assay of DNA but which does not persist to the final suspension of nuclei. Further experiments indicate that nylon bolting cloth is suitable as a filtration material without the above disadvantages. With this nylon bolting cloth the recovery of DNA from the filtered homogenate is almost quantitative (90–100 %). It is certain therefore that a good recovery of nuclei is obtained from the homogenate filtered through flannelette. The quotient,

Table 2. *Diphosphopyridine nucleotide pyrophosphorylase in nuclei isolated from rats after various treatments*

All substances were injected as solutions in 0·9% NaCl solution, except CCl_4 which was as a 10% (v/v) solution in arachis oil. i.v., Intravenous; i.p., intraperitoneal; s.c., subcutaneous; oral, stomach tube.

Treatment and reference	Dose (mg./kg. body wt.)	Time killed	Protein in nuclear fraction (mg./g. wet wt. of perfused liver)	*P*	DPN pyrophosphorylase in nuclear fraction (μmole of DPN/mg. of protein/hr.)	*P*
Control	—	—	7·4±0·99 (5)	—	0·228±0·026 (5)	—
Beryllium (*a*)	0·6 (i.v.)	24 hr.	8·0±0·85 (4)	0·4	0·170±0·037 (4)	0·03
Dimethylnitrosamine (*b*)	50 (i.p.)	24 hr.	7·3±1·0 (4)	0·9	0·206±0·024 (4)	0·2
CCl_4 (*c*)	160 (oral)	24 hr.	7·1±1·1 (4)	0·7	0·189±0·041 (4)	0·13
Ethionine (*d*)	187 (i.p. twice daily)	24 hr.	7·6±2·2 (4)	0·8	0·208±0·052 (4)	0·5
Thioacetamide (*e*)	30 (s.c. daily)	12–13 days	5·2±0·75 (4)	0·01	0·224±0·024 (4)	0·8
	30 (s.c. daily)	18 days	6·4 (2)*	—	0·233 (2)	—
Nicotinamide (*f*)	500 (i.p.)	3 hr.	6·3 (2)	—	0·226 (2)	—
	500 (i.p.)	12 hr.	7·8±0·4 (3)	0·6	0·204±0·022 (3)	0·9
Partial hepatectomy (*g*)	—	24 hr.	4·0±0·5 (4)	<0·001	0·185±0·016 (4)	0·02
	—	48 hr.	5·4±1·3 (4)	0·04	0·147±0·013 (4)	<0·001
	—	72 hr.	5·1±0·76 (4)	0·006	0·106±0·019 (4)	<0·001

* This value is the mean of 4·6 and 8·3. The latter is undoubtedly too high owing to a loss of diluting fluid during isolation. This will not, however, influence the specific-activity measurements.

(*a*) Aldridge, Barnes & Denz (1949), Cheng (1956). (*b*) Barnes & Magee (1954). (*c*) Cameron & Karunaratne (1936). (*d*) Koch-Weser, Farber & Popper (1951). (*e*) Gupta (1956). (*f*) Bonsignore & Ricci (1958), Kaplan, Goldin, Humphreys, Ciotti & Stolzenbach (1956). (*g*) Higgins & Anderson (1931).

protein/DNA P (mg. of protein as albumin/mg. of DNA P), is 49 for the nuclear fraction.

Diphosphopyridine nucleotide pyrophosphorylase in nuclei from livers of rats after various treatments. In Table 2 are the results of assays of DPN phosphorylase in the nuclear fractions isolated from animals after various treatments.

DISCUSSION

For studies of this kind, nuclei should be obtained from liver in the state in which they are present in the liver cells. To achieve this perfection may be an impossibility but, unlike our knowledge of mitochondria (Aldridge, 1957), we have little or no knowledge of what criteria to adopt in order to judge the state of our preparations. The procedure in this paper has been to test our preparations with properties that have been suggested by various authors. Qualitatively this preparation seems to fulfil these criteria. Many treatments of nuclei cause them to lose protein thus lowering the quotient, protein/DNA P (Allfrey *et al.* 1952). Chauveau, Moulé & Rouiller (1956) obtained a value of 40 for nuclei isolated by high-density sucrose solutions, whereas values of 61 and 78 were obtained for calf- and horse-liver nuclei respectively with the non-aqueous method (Allfrey *et al.* 1952). The value for our preparation of approx. 49 falls between these values. However, the high values obtained by the latter authors were calculated by using a value for protein obtained by subtracting the DNA and RNA from the total dry weight.

DPN-pyrophosphorylase activity in the isolated nuclei has been determined by the formation of DPN from nicotinamide mononucleotide and ATP. However, recent work has indicated that DPN is not formed in this way *in vivo*. Priess & Handler (1958*a*) have shown that, in human erythrocytes and in rat liver *in vivo*, after the administration of nicotinic acid the intermediates found are nicotinic acid mononucleotide and deamido-DPN. These intermediates have also been found after the administration of nicotinamide (Langan, Kaplan & Shuster, 1959; Threlfall, 1959). At the enzymic level we have to consider whether the reactions whereby nicotinamide mononucleotide is converted into DPN and nicotinic acid mononucleotide is converted into deamido-DPN are catalysed by the same enzyme. Nicotinic acid mononucleotide-pyrophosphorylase and DPN-pyrophosphorylase activities are closely associated at all stages of Kornberg's (1950) method of purification (Priess & Handler, 1958*b*), and the distribution of the two activities between the various subcellular fractions from rat liver is also very similar (Priess & Handler, 1958*b*). The available evidence therefore indicates that the enzymic activity measured in this work is the

same enzyme which converts nicotinic acid mononucleotide into deamido-DPN *in vivo*. Therefore, contrary to the scheme put forward by Morton (1958), the enzyme measured is not now the terminal enzyme in the synthesis of DPN *in vivo*. At the final enzymic step deamido-DPN is converted into DPN.

Obvious damage, including nuclear changes, is present in rat liver 24 hr. after the administration of dimethylnitrosamine (Barnes & Magee, 1954), carbon tetrachloride (Cameron & Karunaratne, 1936) and beryllium (Aldridge, Barnes & Denz, 1949; Cheng, 1956). After the single doses of ethionine, fatty livers are found without any necrotic changes (Koch-Weser, Farber & Popper, 1951). After 14–21 daily doses of thioacetamide the nuclei in many of the liver cells are almost double their normal size with the single nucleolus almost as big as a normal-size nucleus (Gupta, 1956). It is particularly striking that in this work we have found little change in the DPN-pyrophosphorylase activity of the nuclei isolated from livers of animals so treated. Only after injection of beryllium is the change statistically significant (P 0·03); but these are marginal changes, particularly since Cheng (1956) has reported that changes in the nuclei were visible 5 hr. after dosing. Even with grossly abnormal nuclei (after injection of thioacetamide) there is no change in the specific activity of the nuclei. These results indicate that this enzyme is not suitable as an indicator of nuclear damage or change, for it is apparent that such morphological changes are not accompanied by changes in enzyme activity. This situation may be compared with mitochondria and succinic dehydrogenase. This enzyme is very stable and so firmly attached to the mitochondrial structure that no early changes are to be expected with mitochondrial damage. An example of the contrary situation with change in enzyme activity in nuclei with presumably no morphological changes has been reported. The DPN-pyrophosphorylase activity of livers of mice (Waravdekar, Powers & Leiter, 1956*a*, *b*) and rats (Ono & Tomaru, 1959) bearing tumours at other sites is much lower than that of controls. This contrasts with the normal DPN concentration in the livers from tumour-bearing rats (Glock & McLean, 1957).

After administration of dimethylnitrosamine the DPN pyrophosphorylase of liver nuclei is normal. C. J. Threlfall (unpublished work) has shown that after dimethylnitrosamine the DPN content of the liver falls rapidly to 50% of the control value. However, after injection of nicotinamide, the DPN content of the liver rises by the same absolute amount in both treated and control animals. This indicates that DPN synthesis is unaffected in animals after dimethylnitrosamine and our results are consistent with this view.

After partial hepatectomy the DPN-pyrophosphorylase activity of the nuclei isolated from the regenerating liver decreased continuously for 3 days after operation. During this period the liver is increasing in size and reaches its original weight in 7 days (Abercrombie & Harkness, 1951). A similar low DPN pyrophosphorylase has been found in foetal mouse liver and in liver during the rapid growth after birth (Branster & Morton, 1956). There are few values for the DPN contents of either foetal or regenerating liver with which to compare these results. In the rat low values in embryonic liver (30% of the concentration in adult liver) and a continuously rising content up to normal adult values in 7 days have been reported (Jedeikin, Thomas & Weinhouse, 1956). The concentrations of DPN in regenerating liver examined 3 days after partial hepatectomy were 20% lower than the controls. De Burgh (1957) has reported that, although there was no consistent change in the DPN concentration in regenerating mouse liver, after the injection of nicotinamide the increase in DPN in the liver was 65 and 39% of the controls 1 and 2 days respectively after the removal of the liver. Neglecting the difference in species, these latter results show the same trend as ours.

After partial hepatectomy the remaining liver increases to double its weight in roughly 3 days (Abercrombie & Harkness, 1951; Hammersten, Åqvist, Anderson & Eliasson, 1956; Nygaard & Rusch, 1955). With the results of Abercrombie & Harkness (1951), the total enzyme activity remaining in the liver after operation and for each succeeding day may be calculated. The results are necessarily approximate and depend on the assumption that the rate of regeneration is the same as that obtained by Abercrombie & Harkness (1951) and also that the percentage recovery of nuclei is the same for each preparation. The total enzyme activity obtained by such a calculation is less on all 3 days after operation than that present in the liver remaining immediately after operation. This is a surprising result and indicates that no synthesis of enzyme (other than that necessitated by normal turnover) has occurred. In contrast, DNA in regenerating liver follows the protein and liver weight, both of which double in 3 days (Åqvist & Anderson, 1956; Hecht & Potter, 1956). The activity of many enzymes in regenerating rat liver has been measured (Harkness, 1961), but with few exceptions unfractionated liver homogenates were used. One exception, the cathepsin activity of isolated liver nuclei during regeneration, has been studied (Maver, Greco, Løvtrup & Dalton, 1952). In contrast with DPN pyrophosphorylase the specific activity of the nuclei was considerably higher than that of the controls, 2 and 3 days after operation.

SUMMARY

1. The procedure of Hogeboom *et al.* (1952) for the isolation of liver nuclei has been modified.

2. Some properties of the isolated nuclei have been examined. They do not catalyse a [^{32}P]phosphate–adenosine triphosphate exchange reaction but they do contain more adenosine triphosphatase activity than can be explained by contamination with mitochondria.

3. Little change was found in the specific activity of diphosphopyridine nucleotide pyrophosphorylase of nuclei isolated from livers of rats poisoned with the hepatotoxic agents beryllium, carbon tetrachloride, dimethylnitrosamine and ethionine.

4. After partial hepatectomy, the specific activity of diphosphopyridine nucleotide pyrophosphorylase of nuclei isolated from the regenerating liver falls continuously for 3 days to approximately half of its control value.

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