activity of diphosphopyridinenucleotidase is greatly increased (Nason *et al.* 1951)—there is no reason to attribute invariably the disorders of metabolism which occur in zinc deficiency to a primary impairment of protein synthesis. Copper deficiency has been claimed to have marked effects on the protein metabolism of green plants (Gilbert, Sell & Drosdoff, 1946; Wood & Womersley, 1946; Lucas, 1948; Gilbert, 1951), and so the observation that the aldolase activity in the leaves of copper-deficient oats remains unimpaired, suggests that the lowered aldolase activity in the leaves of zinc-deficient oats is not due primarily to a decreased protein synthesis; although zinc might be specifically involved in the synthesis of the enzyme itself.

The third possibility that the aldolases of green leaves are activated by Zn^{2+} or by zinc-containing co-enzymes is being examined.

SUMMARY

Aldolase activity was found to be materially decreased in tissue suspensions prepared from the leaves of zinc-deficient plants of *Trifolium subterraneum* and of *Avena sativa*, but was unchanged by copper deficiency.

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The Deoxyribonucleic Acid Content of the Rat Cell Nucleus and its Use in Expressing the Results of Tissue Analysis, with Particular Reference to the Composition of Liver Tissue

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In 1948, Boivin, Vendrely & Vendrely published figures for the deoxyribonucleic acid (DNA) content of the cell nucleus of vertebrates, based on the chemical analysis of a known number of isolated nuclei, and claimed that the amount of DNA per nucleus was constant for the somatic cells of different tissues and was approximately double that found in the haploid sperm cells. On the other hand, a considerable variation occurred between species, birds and fish having, in general, a lower DNA content per nucleus than mammals (Vendrely & Vendrely, 1948, 1949). The general trend of these

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observations was confirmed by Mirsky & Ris (1949) and for fowl tissues, by Davidson, Leslie, Smellie & Thomson (1951).

The original observations of the Vendrelys were apparently made on very small numbers of animals and did not include the rat. No systematic investigation of rat tissues appears to have been made apart from the few instances listed in the discussion below.

The results of Vendrely & Vendrely (1948, 1949) are average figures obtained by the gross chemical analysis of large numbers of isolated nuclei, but attempts have also been made to determine the DNA content of individual nuclei by quantitative photometric cytochemical methods employing the Feulgen reaction, methyl green staining or ultraviolet absorption (Ris & Mirsky, 1949; Pollister, 1950; Swift, 1950*a*, *b*; Kurnick, 1950; Pasteels & Lison, 1950 *a–c*; Mirsky & Ris, 1951; Pollister, Swift & Alfert, 1951; Frazer & Davidson, 1952). Such cytochemical methods give comparative figures rather than absolute amounts, but a comparison of the amounts of DNA in isolated nuclei as determined by chemical analysis and by photometric cytochemical analysis has been made by Leuchtenberger, Vendrely & Vendrely (1951) and by Leuchtenberger, Leuchtenberger, Vendrely & Vendrely (1952).

If the DNA content of the cell nucleus is indeed constant for the different somatic tissues of the same animal and for different individuals of the same species, the implications are far-reaching. They have already been discussed by Davidson & Leslie (1950 *a*, *b*), by Mirsky & Ris (1951) and by Davidson (1952). Since information concerning the composition of rat nuclei is limited and since the rat is so commonly employed in laboratory investigations, we have thought it desirable to examine the DNA content of rat cell nuclei from reasonably large numbers of animals under different conditions. We have, moreover, paid particular attention to the rat liver, which is so extensively used in biochemical work and in which the picture is complicated by the presence of polyploid and binucleate cells.

A brief preliminary account has already been published (Thomson, Heagy, Hutchison & Davidson, 1952).

EXPERIMENTAL

Animals. Except where otherwise stated, the experiments were carried out on male albino rats (wt. 190–250 g.) from the departmental colony. Where indicated in the tables, male albino rats outside this weight range, female albino, and male and female hooded rats were also used.

Diets. The diets employed were as follows.

(*a*) A stock diet of 'rat cake' made by Levers Cattlefoods Ltd.

(*b*) A semi-synthetic diet similar to that used in the University of Illinois consisting of glucose, 73%; vitamin-free casein, 18%; salt mixture, 4%; arachis oil, 5%. Each kg. of diet contained the following supplement: thiamine hydrochloride, 2·5 mg.; riboflavin, 5·0 mg.; nicotinic acid, 10·0 mg.; pyridoxin hydrochloride, 2·5 mg.; calcium pantothenate, 20·0 mg.; inositol, 100·0 mg.; *p*-aminobenzoic acid, 50·0 mg.; biotin, 0·1 mg.; folic acid, 1·0 mg.; 2-methylnaphthaquinone, 1·0 mg.; choline chloride, 1·0 g. Each animal received one drop weekly of a mixture of 1 g. α-tocopherol in 14 g. Radiostoleum (British Drug Houses Ltd.).

The salt mixture employed was that described by Griffin, Nye, Noda & Luck (1948).

(*c*) A protein-free diet similar to (*b*) above in which casein was replaced by glucose and additional phosphate was supplied as recommended by Kosterlitz (1947).

(*d*) A thiamine-deficient diet similar to (*b*) above but without thiamine hydrochloride.

(*e*) A thioacetamide-containing diet similar to (*b*) above but containing in addition 0·032% thioacetamide.

(*f*) A high-fat diet as described by Channon, Mills & Platt (1943) containing casein, 8%; beef fat, 40%; glucose, 46%; salt mixture, 5%; cod liver oil, 1%. Each rat received 10 μg. thiamine per day.

(*g*) A carcinogenic diet similar to that used by Griffin *et al.* (1948) containing casein, 18%; glucose, 73%; arachis oil, 5%; salt mixture, 4%; *p*-dimethylaminoazobenzene, 0·06%, and vitamin supplement. Control animals were kept for the same time on the same diet without the dimethylaminoazobenzene.

Treatment of animals. Some animals were subjected to the procedure of partial hepatectomy (removal of median and left lateral lobes), by the method of Higgins & Anderson (1931).

Others received alloxan as described by Diermeier, Di Stephano, Tepperman & Bass (1951). Such animals were fasted for 48 hr. before receiving, by subcutaneous injection, 175 mg. alloxan monohydrate/kg. body wt. Control animals received injections of saline. The development of diabetes was confirmed by blood-sugar estimations. Animals which did not develop diabetes were discarded.

Isolation of nuclei. All rats were killed by exsanguination under ether anaesthesia. The livers and other organs required for analysis were quickly excised, weighed and finely chopped with scissors. A small piece of tissue was taken for histological examination, about 0·5 g. was used for whole-tissue analysis, and the remainder used for isolation of nuclei by a modification of the citric acid procedure as employed by Mirsky & Pollister (1946). When the tissues could not be treated immediately they were preserved by freezing in solid CO_2.

The tissues were homogenized in a Waring, Atomix or Nelco Blendor, and without further use of the Blendor the nuclei were separated from the homogenates by differential centrifugation in a refrigerated centrifuge. The isolation procedure was checked microscopically as required. The number of nuclei in the final suspension was determined by counting in a haemocytometer chamber using 0·01 M-citric acid as diluting fluid. At least 1600 nuclei were counted by two observers working independently.

Leucocytes were isolated from the pooled citrated blood of about twelve rats by centrifuging in constricted centrifuge tubes according to the method used by Butler & Cushman (1940). They were suspended in 0·01 M-citric acid and counted in a haemocytometer in the usual way.

Methods of analysis. For whole-tissue analysis the method of Schmidt & Thannhauser (1945) as modified by Leslie & Davidson (1951) was followed. Phosphorus was determined

by the method of Allen (1940) in the lipid fraction, the DNA fraction and the ribonucleic acid (RNA) fraction. This last fraction contains not only nucleotide P derived from the breakdown of RNA but also small amounts of inorganic P derived from 'phosphoprotein' (Davidson, Frazer & Hutchison, 1951) and small amounts of non-nucleotide organic P (Smellie & Davidson, 1951; Davidson & Smellie, 1952). Figures for RNAP must therefore be regarded as approximate. In one experiment the individual nucleotides in the RNA fraction were determined by the paper-ionophoresis method of Davidson & Smellie (1952).

Protein N was determined by estimating the N content of a portion of the alkaline digest of the tissue residue from which acid-soluble and lipid material had been removed, and subtracting the calculated amount of nucleic acid N.

A portion of the nuclear suspension containing a known number of nuclei was treated with 0·5 vol. 30% (w/v) trichloroacetic acid (TCA) and submitted to the modified fractionation procedure of Schmidt & Thannhauser (1945). The precipitate of the fraction containing DNA and protein was washed with a few drops of ice-cold distilled water to remove TCA and was then dissolved in NaOH to a final concentration of 0·2 N. Portions of this solution were used for determination of (*a*) P by Allen's method, (*b*) deoxypentose by the diphenylamine method (Davidson & Waymouth, 1944) and (*c*) ultraviolet absorption calculated from the difference between the absorptions at 290 and 260 mμ. as measured in a Beckman DU spectrophotometer or a Unicam SP500 quartz spectrophotometer. The diphenylamine and ultraviolet methods were calibrated with a standard prepared from thymus DNA. In this way three separate determinations were made of DNA based on phosphorus, deoxypentose and ultraviolet absorption. In all cases the results were expressed in terms of DNAP.

Significance tests. Student's '*t*' test was used to assess the significance of the difference between the mean values of two groups of data. When more than two groups were compared analysis of variance (Snedecor, 1946) was used. The conventional notation $P < 0.05$ and $P < 0.01$ is used below to indicate significance on the 5 and 1% levels respectively.

RESULTS

DNA content/nucleus in different tissues. The mean values for the DNA content of the nuclei of various rat tissues are shown in Table 1 expressed in terms of picograms (pg.) DNAP/nucleus (1 pg. = 10^{-12} g.). The agreement between the three methods of estimation is reasonably good, although figures obtained by the deoxypentose estimation tend to be higher than those found by the other two methods.

The values obtained for the non-hepatic tissues are of the order of 0·65–0·70 pg. DNAP/nucleus (equivalent to 6·7–7·2 pg. DNA), although small intestine in both young and adult rats and salivary gland and pancreas in adult rats give slightly higher values. Analysis of variance, however, shows that no matter which of the three methods of estimating DNA is used there is no significant difference between the mean values found for the different non-hepatic tissues of either the young or the adult rat.

Liver nuclei from adult rats, on the other hand, give a value of the order of 0·9 pg. DNAP (equivalent to 9·3 pg. DNA). The corresponding figures for young rats and for embryos are, however, much lower and closer to those for the non-hepatic tissues.

DNA content/nucleus in liver tissue. Table 2, section 1, shows the effect of sex, strain, body weight and pregnancy on the DNAP content of the liver nuclei. The mean values for the different groups of animals fall very close together, and analysis of variance for each of the three methods of estimation does not indicate that the slight differences between them are significant.

The effect of various dietary treatments on the average DNAP content of the liver nuclei is shown in Table 2, section 2. Once again analysis of variance for each of the three methods of estimation does not indicate that the small differences between the means for the different groups of animals are significant.

The effect of a diabetogenic dose of alloxan is shown in Table 2, section 3. The values of '*t*' found for each of the three methods of estimation do not indicate a significant change in the average DNAP content of the liver nuclei although Diermeier *et al.* (1951) reported that, in the rat, alloxan caused a 12% increase in the DNA content of round liver nuclei of about 7 μ. diameter.

RNA content of isolated nuclei. Figures for the average RNA content of the isolated nuclei are also presented in Tables 1 and 2. In Table 1 they are expressed as a range since only a few results were available for each tissue and these showed a wide scatter. The results obtained for liver nuclei were more numerous and consistent and are therefore expressed as mean values with standard errors. They must, however, be interpreted with considerable caution since, although nuclei are known to contain some RNA (McIndoe & Davidson, 1952), this constitutes only a small percentage of the total RNA of the tissue. The apparent RNA content of isolated nuclei will therefore be substantially increased if they are contaminated with cytoplasmic debris. Nevertheless, it is of interest to observe that fasting appears to cause a decrease in the average RNA content of the liver nuclei and that (in agreement with Laird, 1952) thioacetamide causes an increase.

Composition of whole liver tissue. The results of the analyses of whole liver tissue are shown in Table 3 expressed as (*a*) concentrations/100 g. fresh liver, (*b*) total amounts in mg./liver and (*c*) pg./pg. DNAP. Since the average DNAP content/nucleus in the liver of the adult rat is about 0·9 pg. irrespective of its sex, strain and body weight, and of the diet on which it has been maintained, the third method of expression gives an approximate estimate of the average cell composition. Similarly, the total

DNAP content of the liver in pg. gives an approximate estimate of the number of cells which it contains. Both these estimates are approximate unless corrections are made for the occurrence of binucleate cells in the liver and for the fact that a proportion of the liver substance is extracellular.

The usefulness of this method of expression may be appreciated from a consideration of the differences in liver composition between male and female rats of the same strain and of comparable body weight (Table 3, section 1). It will be seen that in both the albino and hooded strains there is little difference between the sexes in the concentrations of lipid phosphorus (LP), protein nitrogen (PN), or RNAP/100 g. liver. The concentration of DNAP, on the other hand, is about 20 % higher in females than in males and this difference is significant ($P < 0{\cdot}01$ in both strains). A similar difference has been observed by Lowe & Salmon (1951). The total amount of LP/liver is significantly lower in females than in males ($P < 0{\cdot}01$ for both strains) but the differences in the total amounts/liver of PN, RNAP and DNAP are of dubious significance and are not consistent between the two strains. When, however, the results are referred to DNAP it becomes clear that, in the female, the average mass of the cell and its average

Table 1. *Mean values for the nucleic acid content, in terms of deoxyribonucleic acid phosphorus (DNAP) and ribonucleic acid phosphorus (RNAP), of cell nuclei isolated from tissues of male albino rats on stock diet*

(Results are given ± S.E. The figures in brackets represent the number of observations. Adult rats weighed 195–250 g.; young rats weighed 35–90 g.)

Tissue	Stage of development of animals	DNAP (pg./nucleus) as determined by: Phosphorus estimation	Deoxypentose estimation	Ultraviolet absorption	Range of RNAP (pg./nucleus)
Kidney	Adult	0·652 ± 0·0202 (8)	0·664 ± 0·0284 (7)	0·663 ± 0·0145 (8)	0·088–0·211
	Young	0·654 ± 0·0167 (3)	0·702 ± 0·0440 (2)	0·636 ± 0·0077 (3)	0·055–0·139
Spleen	Adult	0·633 ± 0·0247 (8)	0·662 ± 0·0454 (8)	0·634 ± 0·0281 (8)	0·057–0·156
	Young	0·685 ± 0·0278 (3)	0·726 ± 0·0040 (2)	0·694 ± 0·0455 (3)	0·055–0·163
Lung	Adult	0·651 ± 0·0311 (6)	0·692 ± 0·0388 (5)	0·647 ± 0·0283 (6)	0·031–0·262
	Young	0·595 (1)	—	0·595 (1)	0·067
Small intestine	Adult	0·738 ± 0·0175 (4)	0·776 ± 0·0157 (4)	0·684 ± 0·0250 (4)	0·136–0·191
	Young	0·728 (1)	—	0·712 (1)	0·244
Salivary gland	Adult	0·733 ± 0·0170 (2)	—	0·663 ± 0·0110 (2)	0·106–0·258
	Young	0·637 (1)	—	0·626 (1)	0·208
Leucocytes	Adult	0·641 ± 0·0508 (4)	—	0·661 (1)	—
Heart	Adult	0·627 (1)	—	0·689 (1)	—
Bone marrow	Adult	0·670 (1)	—	—	—
Pancreas	Adult	0·712 ± 0·0010 (2)	—	0·726 ± 0·0050 (2)	—
Thymus	Young	0·718 ± 0·0215 (4)	0·719 ± 0·0450 (4)	0·660 ± 0·0365 (4)	0·050–0·127
Liver	Adult	0·913 ± 0·0115 (38)	0·928 ± 0·0190 (30)	0·870 ± 0·0157 (38)	0·103–0·232
	Young	0·758 ± 0·0198 (13)	0·788 ± 0·0267 (11)	0·759 ± 0·0152 (13)	0·134–0·251
	Embryo	0·780 ± 0·0717 (4)	0·841 ± 0·0850 (4)	0·721 ± 0·0763 (4)	0·108

Table 2. *Mean values for the nucleic acid content, in terms of deoxyribonucleic acid phosphorus (DNAP) and ribonucleic acid phosphorus (RNAP), of rat liver nuclei*

(Results are given ± S.E. except where stated. The figures in brackets represent the number of observations.)

Treatment of animal	Strain and sex	Body wt. (g. ± S.D.) Initial	Body wt. (g. ± S.D.) Final	DNAP (pg./nucleus) as determined by: Phosphorus estimation	Deoxypentose estimation	Ultraviolet absorption	RNAP (pg./nucleus)
Section 1:							
All animals on stock diet (*a*)	Albino, M.	—	221 ± 14 (43)	0·913 ± 0·0115 (38)	0·928 ± 0·0189 (30)	0·870 ± 0·0157 (38)	0·180 ± 0·00639 (23)
	Albino, M.	—	271 ± 12 (14)	0·873 ± 0·0287 (14)	0·861 ± 0·0382 (11)	0·844 ± 0·0294 (12)	0·208 ± 0·0343 (5)
	Albino, F.	—	199 ± 11 (14)	0·942 ± 0·0397 (10)	1·004 ± 0·0378 (6)	0·912 ± 0·0557 (9)	0·172 ± 0·0117 (9)
	Albino, F. (pregnant)	—	234 ± 17 (12)	0·893 ± 0·0222 (12)	0·919 ± 0·0396 (10)	0·832 ± 0·0275 (12)	0·181 ± 0·0134 (3)
	Hooded, M.	—	215 ± 12 (12)	0·921 ± 0·0248 (12)	0·965 ± 0·0371 (12)	0·855 ± 0·0274 (12)	0·182 ± 0·0148 (10)
	Hooded, M.	—	291 ± 15 (13)	0·903 ± 0·0316 (13)	0·942 ± 0·0352 (13)	0·851 ± 0·0287 (13)	0·188 ± 0·0881 (12)
	Hooded, F.	—	215 ± 13 (11)	0·931 ± 0·0333 (10)	0·951 ± 0·0606 (10)	0·890 ± 0·0567 (10)	0·169 ± 0·0850 (7)
Section 2. Albino rats of initial wt. 200–250 g. on various diets:							
Stock diet (*a*)	Albino, M.	—	221 ± 14 (43)	0·913 ± 0·0115 (38)	0·928 ± 0·0189 (30)	0·870 ± 0·0157 (38)	0·180 ± 0·00639 (23)
Fast (72 hr.) after diet (*a*)	Albino, M.	224 ± 12 (7)	190 ± 11 (7)	0·885 ± 0·0392 (6)	1·026 ± 0·0210 (3)	0·860 ± 0·0245 (5)	0·144 ± 0·0134 (6)
High-fat diet for 14 days	Albino, M.	223 ± 7 (17)	198 ± 17 (17)	0·889 ± 0·0229 (15)	0·946 ± 0·0372 (10)	0·871 ± 0·0293 (14)	0·169 ± 0·0103 (11)
High-fat diet for 35 days	Albino, M.	217 ± 6 (3)	184 ± 11 (3)	0·902 ± 0·0315 (3)	0·937 ± 0·0547 (3)	0·848 ± 0·0313 (3)	0·160 ± 0·00658 (3)
Semi-synthetic diet (*b*)	Albino, M.	—	223 ± 12 (16)	0·893 ± 0·0294 (12)	0·919 ± 0·0304 (11)	0·852 ± 0·0279 (12)	0·173 ± 0·0200 (6)
Fast (48 hr.) after diet (*b*)	Albino, M.	219 ± 7 (14)	205 ± 11 (14)	0·916 ± 0·0191 (13)	0·943 ± 0·0280 (10)	0·883 ± 0·0244 (13)	0·155 ± 0·0104 (7)
Protein-free diet (*c*) for 7 days	Albino, M.	223 ± 6 (13)	199 ± 10 (13)	0·943 ± 0·0386 (9)	0·990 ± 0·0628 (7)	0·908 ± 0·0424 (9)	0·197 ± 0·0152 (8)
Protein-free diet (*c*) for 15 days	Albino, M.	217 ± 8 (12)	180 ± 15 (12)	0·872 ± 0·0308 (12)	0·897 ± 0·0366 (11)	0·837 ± 0·0320 (12)	0·183 ± 0·0154 (8)
Thiamine-deficient diet (*d*) for 21 days	Albino, M.	228 ± 20 (10)	196 ± 17 (10)	0·912 ± 0·0380 (7)	0·917 ± 0·0594 (6)	0·868 ± 0·0376 (7)	0·141 ± 0·0119 (4)
Thioacetamide-containing diet (*e*) for 7 days	Albino, M.	215 ± 12 (4)	204 ± 18 (4)	0·917 ± 0·0582 (4)	0·934 ± 0·0748 (3)	0·894 ± 0·0683 (4)	0·263 ± 0·0314 (4)
Section 3:							
Alloxan-diabetic rats	Albino, M.	216 ± 8 (5)	187 ± 16 (5)	0·891 ± 0·0125 (5)	0·976 ± 0·0346 (4)	0·888 ± 0·0134 (5)	0·147 ± 0·00734 (5)
Controls for alloxan-diabetic rats	Albino, M.	212 ± 2 (4)	213 ± 6 (4)	0·874 ± 0·0305 (4)	0·886 ± 0·0365 (3)	0·879 ± 0·0332 (4)	0·147 ± 0·00805 (4)

content of LP, PN and RNAP are 15–30 % lower than in the male. Similar calculations show that in pregnancy (Table 3, section 2) the total amount of DNAP (i.e. number of cells) in the liver is not significantly altered but the average cell mass increases by 12 % ($P < 0.02$), the average cell content of LP by 20 % and of RNAP by 30 %. No significance can be attached to the increase in average cell content of PN. These results are in agreement with the finding of Campbell & Kosterlitz (1949) that in pregnancy there is a marked increase in the total RNA content of the liver.

Some figures for the composition of liver tissue from young animals and embryos are included in Table 3, section 2. The liver of the young animal has a composition not unlike that of the adult, but the embryo liver contains about twice as much DNAP and only half as much LP/100 g. fresh wt. Making allowance for the fact that the DNAP content/nucleus in this tissue is lower than in adult liver (0·75 pg. compared with 0·90 pg.) it would appear that the cells in the embryo liver are only half as large as those in the adult organ and that they contain only half as much RNAP and a quarter as much LP.

Effects of various diets. In considering the effects of the various dietary treatments shown in sections 3 and 4 of Table 3, it is convenient to consider separately their effect on the total number of cells in the liver and on the average cell composition. It is obvious that none of the treatments has had any marked effect on the total amount of DNAP/liver. The small differences between the means for the different groups have been found by analysis of variance to be non-significant. Therefore it appears that none of the treatments has significantly affected the total number of cells in the liver. The average cell composition, on the other hand, is drastically changed. On fasting after either the semi-synthetic or the stock diet, the average cell mass falls by about 35 % and the average cell content of LP, PN and RNAP by 20–30 %. In an experiment in which the nucleotides were separated from the RNA fraction by paper ionophoresis it was found that they accounted for 72·1 and 68·5 % of the phosphorus in this fraction in the livers of two fed rats and for 72·9 and 70·0 % in the livers of two rats fasted for 72 hr. It is clear from this observation that the amounts of nucleotides and of the other phosphorus-containing compounds in this fraction are about equally affected by fasting.

The thiamine-deficient diet produced results similar to those of fasting which may, however, be due not so much to the vitamin deficiency as to the resulting loss of appetite. The protein-free diet caused a 20 % fall in the average cell mass and a 30 % fall in the average cell content of LP, PN and RNAP during the first 7 days. During the second 7 days there was a further significant loss of LP, PN and cell mass ($P < 0.01$, $P < 0.02$, $P < 0.01$ respectively) but the RNAP content did not decline further. The effects of the high-fat diet on the LP, PN and RNAP content of the cell were similar to those of protein deficiency, but less severe. This might be expected since the high-fat diet contains only 8 % protein. The average cell mass did not fall, and it is presumed that increased deposition of neutral fat balanced the loss of other cytoplasmic constituents.

Although our histological observations agree with those of Rather (1951), who found that thioacetamide increases the volume of the liver nuclei, this substance does not appear to have any effect on the composition of whole liver tissue. Similar findings have been recorded by Laird (1952).

Finally, the animals on the stock diet differed from those on the semi-synthetic diet in having a significantly higher content of LP/cell in the liver ($P < 0.01$). The difference disappeared on fasting.

Administration of a diabetogenic dose of alloxan (Table 3, section 5) caused a significant fall in the weight of the liver ($P < 0.01$) and in its total content of LP and RNAP ($P < 0.01$ and $P < 0.02$ respectively). Since, however, the falls in total content of DNAP (i.e. in number of cells/liver) and in average cell mass and content of LP and RNAP which accompanied these changes are not statistically significant, it is not possible to say whether the changes were due to a reduction of cell number or to a change in average cell mass and composition, or to both.

Effects of carcinogenic diet. The effects of the carcinogenic diet on the average DNAP content of the liver nuclei and the composition of whole liver are shown in Table 4. Compared with the controls, the animals which received the carcinogen had a lower average content of DNAP/nucleus and a higher concentration of DNAP/100 g. In those cases in which the total DNAP content of the liver was determined, it was generally found to be considerably increased. The total amounts and concentrations/100 g. of LP, PN and RNAP in the liver were not greatly affected but the ratios of these components to DNAP were markedly lower. Some animals developed tumours sufficiently large and well defined to be dissected out from the rest of the liver and analysed separately. Where this was done (rats no. H 5, 107–108, and 109–110) it was found that the tumour exhibited to an exaggerated degree the changes described above. The average DNAP content/nucleus was of the same order as the value found for the non-hepatic tissues of the normal animal. The ratios of LP, PN, RNAP and tissue mass to DNAP were only half as great as in the controls. In the non-tumour portion of the liver the average DNAP content/nucleus was also very low but the ratios of LP, PN, RNAP and tissue mass to

Table 3. *Mean composition of rat liver tissue in terms of lipid phosphorus (LP), protein nitrogen (PN),*

(Results are given ± S.E. except where stated. The

Treatment of animal	Strain and sex	Body wt. (g. ± S.D.) Initial	Body wt. (g. ± S.D.) Final	Liver wt. (g.)	LP (mg./100 g. liver)	PN (mg./100 g. liver)	RNAP (mg./100 g. liver)	DNAP (mg./100 g. liver)
Section 1. Stock diet (*a*)	Albino, M.	—	212 ± 8 (24)	7·67 ± 0·190 (23)	132·8 ± 3·31 (23)	2523 ± 64·1 (14)	93·2 ± 1·55 (23)	21·6 ± 0·664 (23)
	Hooded, M.	—	215 ± 12 (12)	7·28 ± 0·262 (12)	142·6 ± 4·30 (11)	2618 ± 55·7 (11)	91·1 ± 1·89 (11)	22·9 ± 0·884 (11)
	Albino, M.	—	276 ± 10 (10)	8·53 ± 0·212 (10)	127·7 ± 3·56 (10)	2401 ± 53·0 (6)	90·3 ± 3·81 (10)	21·0 ± 1·01 (10)
	Hooded, M.	—	281 ± 9 (8)	8·83 ± 0·401 (8)	141·1 ± 4·70 (8)	2545 ± 95·8 (8)	94·0 ± 3·62 (8)	22·2 ± 0·940 (8)
	Albino, F.	—	207 ± 8 (8)	6·55 ± 0·348 (8)	123·9 ± 2·89 (8)	2522 ± 81·3 (3)	99·9 ± 2·80 (8)	27·4 ± 0·479 (8)
	Hooded, F.	—	215 ± 13 (11)	6·96 ± 0·295 (11)	125·5 ± 2·44 (11)	2598 ± 76·5 (11)	99·7 ± 2·98 (11)	27·7 ± 1·26 (11)
Section 2. Stock diet (*a*)	Albino, F.	—	199 ± 11 (14)	6·59 ± 0·266 (14)	127·1 ± 2·14 (14)	2566 ± 55·1 (8)	98·6 ± 1·71 (14)	26·9 ± 0·406 (14)
	Albino, F. (pregnant)	—	234 ± 17 (11)	7·65 ± 0·258 (11)	135·0 ± 3·80 (10)	2531 ± 61·5 (8)	114·9 ± 4·22 (10)	24·4 ± 1·17 (10)
	Albino (embryo)	—	—	—	60·48 ± 3·52 (4)	—	89·9 ± 3·46 (4)	45·9 ± 3·46 (4)
	Albino, M.	—	35–90	—	121·5 ± 3·09 (13)	2358 ± 39·2 (6)	100·0 ± 2·48 (13)	25·6 ± 0·799 (13)
Section 3.								
Stock diet (*a*)	Albino, M.	—	221 ± 14 (43)	7·94 ± 0·149 (43)	131·4 ± 2·24 (41)	2469 ± 47·7 (27)	91·8 ± 1·32 (41)	21·9 ± 0·479 (41)
Fast (72 hr.) after stock diet	Albino, M.	224 ± 12 (7)	190 ± 11 (7)	4·89 ± 0·158 (7)	141·4 ± 2·98 (7)	2955 ± 4·95 (2)	109·1 ± 4·86 (7)	33·4 ± 1·64 (7)
High-fat diet for 14 days	Albino, M.	223 ± 7 (17)	198 ± 17 (17)	9·01 ± 0·455 (17)	89·0 ± 3·14 (17)	1922 ± 74·5 (7)	68·1 ± 2·18 (17)	20·6 ± 0·731 (17)
High-fat diet for 35 days	Albino, M.	217 ± 6 (3)	184 ± 11 (3)	8·40 ± 0·378 (3)	92·1 ± 1·97 (3)	1891 ± 94·0 (3)	67·1 ± 2·38 (3)	20·0 ± 1·43 (3)
Section 4.								
Semi-synthetic diet (*b*)	Albino, M.	—	223 ± 12 (16)	8·22 ± 0·178 (16)	110·5 ± 2·62 (15)	2380 ± 116·0 (9)	91·9 ± 2·44 (15)	21·1 ± 0·702 (15)
Fast (48 hr.) after diet (*b*)	Albino, M.	219 ± 7 (14)	205 ± 11 (14)	5·53 ± 0·179 (14)	139·8 ± 2·30 (13)	2603 ± 82·6 (8)	107·7 ± 2·33 (13)	33·3 ± 0·899 (13)
Protein-free diet (*c*) for 7 days	Albino, M.	223 ± 6 (13)	199 ± 10 (13)	6·30 ± 0·209 (13)	96·8 ± 3·13 (12)	2080 ± 104·1 (8)	81·6 ± 2·37 (12)	25·5 ± 0·650 (12)
Protein-free diet (*c*) for 15 days	Albino, M.	217 ± 8 (12)	180 ± 15 (12)	5·69 ± 0·302 (12)	101·5 ± 1·99 (12)	1845 ± 82·7 (8)	92·2 ± 1·46 (12)	29·4 ± 0·745 (12)
Thiamine-deficient diet for 21 days	Albino, M.	228 ± 20 (10)	196 ± 17 (10)	5·43 ± 0·207 (10)	130·1 ± 6·33 (10)	2945 ± 135·0 (5)	98·0 ± 2·91 (10)	33·9 ± 1·38 (10)
Thioacetamide-containing diet for 7 days	Albino, M.	215 ± 12 (4)	204 ± 18 (4)	7·93 ± 0·688 (4)	111·7 ± 1·68 (3)	2048 ± 19·2 (2)	89·3 ± 2·08 (3)	22·5 ± 0·618 (3)
Section 5.								
Alloxan-diabetic rats	Albino, M.	216 ± 8 (5)	187 ± 16 (5)	6·24 ± 0·300 (5)	128·6 ± 4·38 (5)	—	93·5 ± 4·91 (5)	25·7 ± 0·975 (5)
Controls for alloxan-diabetic rats	Albino, M.	212 ± 2 (4)	213 ± 6 (4)	7·60 ± 0·158 (4)	119·3 ± 3·58 (4)	—	86·2 ± 2·75 (4)	23·2 ± 0·975 (4)

ribonucleic acid phosphorus (RNAP) and deoxyribonucleic acid phosphorus (DNAP)

figures in brackets are the number of observations.)

LP (mg./liver)	PN (mg./liver)	RNAP (mg./liver)	DNAP (mg./liver)	LP (pg./pg. DNAP)	PN (pg./pg. DNAP)	RNAP (pg./pg. DNAP)	Tissue mass (pg./pg. DNAP)
10·21±0·278 (23)	194±5·21 (14)	7·19±0·197 (23)	1·66±0·0534 (23)	6·28±0·264 (23)	120·3±4·93 (14)	4·38±0·120 (23)	4710±130 (23)
10·29±0·415 (11)	189±3·05 (11)	6·55±0·218 (11)	1·63±0·0645 (11)	6·40±0·368 (11)	116·3±6·25 (11)	4·04±0·148 (11)	4480±216 (11)
10·91±0·459 (10)	208±2·24 (6)	7·69±0·338 (10)	1·79±0·0872 (10)	6·22±0·346 (10)	112·5±4·97 (6)	4·32±0·0885 (10)	4880±272 (10)
12·43±0·632 (8)	224±9·86 (8)	8·31±0·546 (8)	1·96±0·120 (8)	6·41±0·249 (8)	115·8±5·79 (8)	4·25±0·112 (8)	4560±186 (8)
8·06±0·318 (8)	158±7·39 (3)	6·56±0·439 (8)	1·80±0·0980 (8)	4·52±0·113 (8)	91·7±5·97 (3)	3·64±0·0880 (8)	3650±61·9 (8)
8·68±0·306 (11)	180±9·25 (11)	6·94±0·363 (11)	1·93±0·116 (11)	4·49±0·235 (11)	92·2±4·51 (11)	3·51±0·0805 (11)	3670±145 (11)
8·33±0·312 (14)	159±8·26 (8)	6·51±0·332 (14)	1·77±0·0698 (14)	4·73±0·109 (14)	95·4±2·93 (8)	3·67±0·0710 (14)	3720±58·0 (14)
10·24±0·372 (10)	192±9·81 (8)	8·69±0·276 (10)	1·84±0·0708 (10)	5·61±0·212 (10)	106·8±4·90 (8)	4·79±0·244 (10)	4180±182 (10)
—	—	—	—	1·33±0·083 (4)	—	1·98±0·140 (4)	2220±161 (4)
—	—	—	—	4·79±0·146 (13)	89·9±2·32 (6)	3·93±0·0805 (13)	3960±137 (13)
10·51±0·213 (41)	198±4·78 (27)	7·35±0·160 (41)	1·75±0·0458 (41)	6·14±0·182 (41)	116·0±3·57 (27)	4·27±0·0925 (41)	4660±93·0 (41)
6·91±0·266 (7)	150±8·49 (2)	5·33±0·292 (7)	1·63±0·0914 (7)	4·30±0·246 (7)	87·1±2·30 (2)	3·28±0·0987 (7)	3030±148 (7)
7·94±0·404 (17)	182±9·22 (7)	6·01±0·206 (17)	1·82±0·0816 (17)	4·27±0·179 (17)	95·4±4·86 (7)	3·33±0·0839 (17)	4960±176 (17)
7·73±0·256 (3)	158±9·24 (3)	5·63±0·277 (3)	1·67±0·109 (3)	4·64±0·226 (3)	95·0±2·38 (3)	3·37±0·124 (3)	5060±358 (3)
9·00±0·229 (15)	193±8·70 (9)	7·53±0·297 (15)	1·73±0·0762 (15)	5·26±0·157 (15)	116·4±7·80 (9)	4·38±0·0890 (15)	4810±160 (15)
7·68±0·200 (13)	146±6·30 (8)	5·93±0·207 (13)	1·83±0·0550 (13)	4·22±0·100 (13)	79·2±3·02 (8)	3·25±0·0631 (13)	3030±84·4 (13)
6·07±0·228 (12)	133±7·89 (8)	5·11±0·143 (12)	1·60±0·0600 (12)	3·79±0·0545 (12)	81·9±6·40 (8)	3·20±0·0488 (12)	3950±102 (12)
5·77±0·323 (12)	108±7·21 (8)	5·22±0·247 (12)	1·67±0·0909 (12)	3·46±0·0845 (12)	62·3±1·80 (8)	3·16±0·0894 (12)	3430±90·2 (8)
7·02±0·337 (10)	153±10·80 (5)	5·31±0·212 (10)	1·83±0·0616 (10)	3·86±0·154 (10)	83·4±8·07 (5)	2·93±0·124 (10)	2990±118 (10)
9·33±0·735 (3)	183±20·5 (2)	7·44±0·480 (3)	1·88±0·1560 (3)	4·98±0·179 (3)	90·3±4·65 (2)	3·98±0·107 (3)	4460±120 (3)
7·98±0·238 (5)	—	5·79±0·206 (5)	1·60±0·0559 (5)	5·01±0·166 (5)	—	3·62±0·0666 (5)	3910±146 (5)
9·05±0·161 (4)	—	6·54±0·176 (4)	1·76±0·0690 (4)	5·16±0·170 (4)	—	3·73±0·0610 (4)	4340±182 (4)

Table 4. *Mean composition of whole tissue and nuclei, in terms of lipid phosphorus (LP), phosphorus (DNAP), in the livers of rats fed*

(Control animals were kept for the same time on the same diet without the dimethylaminoazobenzene. Except

Treatment and description of animals	Rat number	Body wt. at death (g.)	Liver wt. (g.)	Composition of whole liver tissue: LP (mg./100 g. liver)	PN (mg./100 g. liver)	RNAP (mg./100 g. liver)	DNAP (mg./100 g. liver)	LP (mg./liver)	PN (mg./liver)	RNAP (mg./liver)	DNAP (mg./liver)
Carcinogenic diet for 5 months. Initial body wt. 150–170 g.*	H1	132	7·2	—	1950	70·6	32·9	—	140	5·08	2·37
	H2	136	7·8	—	2200L	75·0L	34·8L	—	—	—	—
	H3	105	6·2	—	2000T	79·4T	36·0T	—	—	—	—
	H5	147	13·6	—	—	78·8L	29·2L	—	—	—	—
				—	—	53·8T	39·2T	—	—	—	—
	H6	97	3·3	—	2340	79·0	37·5	—	77	2·60	1·24
	H7	183	8·4	—	1980	63·1	28·0	—	166	5·30	2·35
	H8	139	6·8	—	1990	65·0	32·0	—	135	4·42	2·18
	H9	172	7·5	—	1970	68·7	38·5	—	148	5·15	2·88
	H10	170	9·0	—	2110	75·8	31·4	—	190	6·82	2·83
	H11	132	6·9	—	2170	75·0	34·0	—	150	5·18	2·35
Controls for above: four animals of initial body wt. 163–195 g.*	—	159–199	6·80± 0·791	—	2380± 91	83·3± 2·25	25·5± 0·851	—	161± 16·4	5·64± 0·592	1·73± 0·203
Carcinogenic diet for 6 months. Initial body wt. 190–210 g.	107	180	13·6	77·2T	1950T	72·7T	39·9T	—	—	—	—
	108	200	11·2	105·3L	2260L	76·0L	28·6L	—	—	—	—
	109	195	7·8	63·1T	1570T	68·8T	44·0T	—	—	—	—
	110	155	20·3	96·4L	2160L	64·8L	24·8L	—	—	—	—
	111	210	8·4	104·0	2350	78·6	27·3	8·74	197	6·60	2·29
Controls for above: four animals of initial body wt. 190–210 g.	—	200–243	6·53± 0·441	94·7± 3·83	2440± 38	78·6± 3·36	25·8± 0·477	6·15± 0·291	158± 8·1	5·10± 0·213	1·68± 0·114
Carcinogenic diet for 2 months. Initial body wt. 190–210 g.	465	149	9·2	97·0	—	81·7	37·0	8·92	—	7·52	3·40
	466	141	7·0	98·4	—	85·3	46·7	7·87	—	6·82	3·74
	467	139	6·2	109·5	—	83·3	37·3	6·82	—	5·16	2·31

T, tumour tissue only. L, residual liver tissue after removal of tumour nodules.

DNAP were closer to the normal values. Making allowance for the fact that the average DNAP content/nucleus was lower than in normal liver these observations might be interpreted as follows. The carcinogen caused an increase in the total number of cells in the liver of the order of 50–150 %. The new tumour cells were less than half the average size of the cells of the normal liver. They contained only 35 % as much LP, 35 % as much PN and 40 % as much RNAP.

Effect of hepatectomy. The results of the hepatectomy experiment are shown in Table 5. Throughout the 10 days following the operation the average DNAP content of the nuclei in the remaining lobes of the liver was consistently higher than the average value for unoperated animals of the same sex, strain and age (Table 2). The total amount of DNAP/liver rose steadily for the first 6 days, and during this period there were variations in the average DNAP content of the nuclei and in the ratios of LP and RNAP to DNAP in the whole tissue. From the sixth to the tenth day there was no further increase in the total amount of DNAP and the ratios of LP and RNAP to DNAP likewise showed little change.

DISCUSSION

Our values for the average DNAP content/nucleus in normal adult rat liver (Table 1) are in fairly good agreement with those obtained by other workers using similar methods (Dounce, Tishkoff, Barnett & Freer, 1950; Ely & Ross, 1951*a*; Harrison, 1951; Leuchtenberger *et al.* 1951; Campbell & Kosterlitz, 1952; and Leuchtenberger *et al.* 1952). To calculate the DNA content/liver nucleus Price and his co-workers determined the amount of DNA/100 g. fresh liver and divided this figure by the number of nuclei/100 g., estimated by nuclear counts on a homogenate of a weighed portion of tissue (Price, Miller, Miller & Weber, 1950; Price & Laird, 1950). By this method they found somewhat greater values (10·0–14·0 pg. DNA, equivalent to 0·97–1·36 pg. DNAP). Rose & Schweigert (1952), using the same method, obtained even higher figures (14·9–15·4 pg. DNA, equivalent to 1·45–1·50 pg. DNAP). Although all DNA would be estimated by this method, any nuclei broken during homogenizing would not be counted and the estimated DNA/nucleus would therefore be too high. This is a probable explanation of the high values obtained by these workers. On

protein nitrogen (PN), ribonucleic acid phosphorus (RNAP) and deoxyribonucleic acid a diet containing p-*dimethylaminoazobenzene*

where otherwise indicated analyses and isolation of nuclei were carried out on portions of the whole liver.)

Composition of whole liver tissue				Composition of isolated nuclei				
				DNAP (pg./nucleus) as determined by				
LP (pg./pg. DNAP)	PN (pg./pg. DNAP)	RNAP (pg./pg. DNAP)	Tissue mass (pg./pg. DNAP)	Phosphorus estimation	Deoxypentose estimation	Ultraviolet absorption	RNAP (pg./nucleus)	Remarks
—	59·3	2·15	3040	0·802	0·916	0·774	0·138	Diffuse tumour
—	63·2L	2·16L	2870L	0·722	0·832	0·692	0·143	
—	55·5T	2·21T	2780T	0·792	0·859	0·750	0·133	
—	—	2·70L	3420L	0·792	—	0·744	0·192	
—	—	1·37T	2550T					
—	62·4	2·11	2670	0·823	1·165	0·824	0·121	No obvious tumour
—	70·7	2·25	3570	0·725	0·842	0·669	0·109	Diffuse tumours
—	62·2	2·03	3130	0·721	0·777	0·668	0·105	
—	51·2	1·78	2600	0·700	0·791	0·664	0·108	
—	67·2	2·41	3180	0·715	0·847	0·671	0·119	
—	63·8	2·21	2940	0·731	0·840	0·679	0·096	
—	93·8± 3·85	3·27± 0·082	3940± 126	0·951± 0·0122	0·974± 0·0435	0·907± 0·0499	0·190± 0·0151	Results expressed as means±S.E.
1·93T	48·9T	1·82T	2510T	0·648T	0·643T	0·620T	0·302T	
3·67L	78·8L	2·66L	3500L	0·664L	0·735L	0·713L	0·294L	
1·43T	35·6T	1·56T	2270T	0·651T	—	0·574T	0·428T	
3·89L	87·1L	2·61L	4030L	0·687L	0·710L	0·677L	0·303L	
3·81	94·8	2·88	3660	0·729	0·823	0·718	0·255	Liver almost normal in appearance
3·68± 0·143	94·8± 5·21	3·05± 0·130	3880± 73	0·956± 0·0462	0·947± 0·0339	0·913± 0·0471	0·364± 0·0198	Results expressed as means±S.E.
2·62	—	2·21	2700	0·735	—	0·738	0·136	Diffuse tumours
2·11	—	1·83	2140	—	—	—	—	
2·95	—	2·23	2680	0·732	—	—	0·136	

* Whole tissue analyses carried out by E. B. Smith and G. T. Mills.

the other hand, Cunningham, Griffin & Luck (1950), using their own modification of the citric acid method, found only 5·9–8·0 pg. DNA (equivalent to 0·57–0·78 pg. DNAP)/nucleus in the same organ.

Few results have been published for the nuclei of rat tissues other than liver. For normal intestine Ely & Ross (1951*b*) found that the average DNA content of the nuclei was 6·27 pg. (equivalent to 0·61 pg. DNAP). Cunningham *et al.* (1950) found the average DNA content of thymus nuclei to be 6·1–6·3 pg. (equivalent to 0·58–0·63 pg. DNAP). For kidney, Harrison (1951) and Leuchtenberger *et al.* (1951) found 5·7 and 5·5 pg. DNA (equivalent to 0·53 and 0·55 pg. DNAP) respectively. These observations are in reasonable agreement with those recorded in Table 1. However, in four experiments Mirsky and Kurnick found a value of 8 pg. DNA (equivalent to 0·78 pg. DNAP)/nucleus in both the kidney and liver of the rat (unpublished results quoted by Mirsky & Ris, 1951), and Kurnick (1951) later reported the DNAP content/kidney nucleus as 0·90 pg. (equivalent to 9·3 pg. DNA) for rats ranging in weight from 80 to 219 g.

The results in Table 1 show that with the exception of the liver the average DNA content/nucleus varies within very narrow limits in the rat tissues studied. Liver gives a figure about 30% higher than the other tissues. Ris & Mirsky (1949) and Leuchtenberger *et al.* (1951) have shown by photometric measurements on individual nuclei that the nuclei of the rat liver may be divided into three classes with respect to their content of DNA. Class I nuclei contain the same amount of DNA as the kidney nuclei. Classes II and III contain twice and four times this amount and it is suggested that they are tetraploid and octoploid nuclei. This is in agreement with the histological observations of Beams & King (1942), Sulkin (1943) and McKellar (1949), who found that a considerable proportion of the hepatocytes of rat liver are polyploid, as judged by the criterion of relative nuclear volume. Hence, the average DNAP content/nucleus in the liver depends on the relative proportions of diploid, tetraploid and octoploid nuclei. The lower figures found for the livers of young animals and embryos (Table 1) suggest that they contain a smaller proportion of polyploid nuclei, and McKellar's measurements of nuclear volume in young animals support this view. Swift (1950*a*), too, using the mouse, found by quantitative cytochemical methods

Table 5. *Mean deoxyribonucleic acid phosphorus (DNAP) content of the nuclei of rat liver regenerating after partial hepatectomy and the composition of whole liver tissue in terms of lipid phosphorus (LP), ribonucleic acid phosphorus (RNAP) and deoxyribonucleic acid phosphorus (DNAP)*

(Results given ± S.E. except where stated. Six animals for each time after operation.)

Time after operation (days)	Body wt. (g. ± S.D.)		Composition of whole liver tissue				DNAP (pg./nucleus) as determined by		
	At operation	At death	Liver wt. (g.)	Total DNAP content/liver (mg.)	LP (pg./pg. DNAP)	RNAP (pg./pg. DNAP)	Phosphorus estimation	Deoxypentose estimation	Ultraviolet absorption
1	225 ± 27	220 ± 31	3·02 ± 0·164	0·673 ± 0·0362	5·55 ± 0·204	4·48 ± 0·128	1·018*	1·031*	0·911*
2	232 ± 12	218 ± 8	3·58 ± 0·114	0·769 ± 0·0310	5·91 ± 0·201	4·83 ± 0·208	1·258*	1·355*	1·217*
3	227 ± 22	206 ± 24	3·92 ± 0·282	0·974 ± 0·0463	5·01 ± 0·224	4·98 ± 0·0783	1·022†	0·985†	1·060†
							1·099†	1·019†	1·112†
4	222 ± 35	202 ± 24	5·18 ± 0·364	1·224 ± 0·101	5·29 ± 0·110	4·72 ± 0·154	1·385†	1·375†	1·368†
							1·151†	1·243†	1·138†
6	231 ± 28	216 ± 17	5·42 ± 0·401	1·403 ± 0·0792	4·80 ± 0·184	4·46 ± 0·149	0·903†	0·955†	0·905†
							0·975†	1·015†	0·968†
8	260 ± 33	231 ± 23	5·12 ± 0·273	1·392 ± 0·0952	4·92 ± 0·326	4·61 ± 0·264	1·013†	1·000†	1·061†
							0·949†	0·983†	1·016†
10	228 ± 24	227 ± 12	6·15 ± 0·289	1·445 ± 0·0989	4·61 ± 0·250	4·25 ± 0·271	0·906‡	0·907‡	0·901‡
							1·014‡	1·059‡	1·064‡
							1·056‡	1·200‡	1·047‡

* Pooled tissue from six rats. † Pooled tissue from three rats. ‡ Pooled tissue from two rats.

that the proportion of class II and class III nuclei in the liver is lower in young animals than in adults.

Thus, in the somatic cells of the different tissues of a species, the average amount of DNA in the resting nucleus shows no significant variations in the absence of polyploidy. But in a heteroploid organ like the rat liver the average amount of DNA/nucleus is greater than the diploid value, due to the presence of tetraploid and octoploid nuclei containing two or four times the usual amount of DNA. This is consistent with the view that in the resting nucleus each set of chromosomes contains a constant amount of DNA.

In liver regenerating after partial hepatectomy the average DNAP content/nucleus during the first 4 days was 10–50 % above the mean value found for unoperated animals of the same sex, strain and body weight (Table 5, and Table 2, section 1). This observation is of particular interest because of the extremely high rate of growth and cell multiplication in this tissue (Higgins & Anderson, 1931; Brues, Drury & Brues, 1936; Abercrombie & Harkness, 1951). While mitosis occurs with a frequency of 1 in 7000 to 1 in 20 000 in the liver of a normal adult rat (Brues & Marble, 1937; Marshak & Byron, 1945) the frequency may reach 1 in 40 for parenchymal cells and 1 in 100 for bile-duct cells during the 4 days following partial hepatectomy (Abercrombie & Harkness, 1951). It is obvious that DNA must be synthesized during cell multiplication, but there have been conflicting reports on the relation of DNA synthesis to the stages of the mitotic cycle. According to Swift (1950*a*, *b*), DNA synthesis precedes mitosis, and nuclei that are about to enter mitosis contain a double complement of DNA. In a very rapidly growing tissue many nuclei will be preparing to divide and the average DNA content/nucleus will be increased if this view is correct. Our observations on regenerating liver support Swift's concept. On the other hand, Pasteels & Lison (1950*b*, *c*) concluded that half the normal nuclear content of DNA goes to each daughter nucleus at metaphase, and the full amount/nucleus is restored by synthesis of new DNA during telophase. Such a process would not affect the average DNA content/nucleus unless the nuclear membrane were restored before DNA synthesis were completed, in which case there would be a lower average DNA content/nucleus. Our observations of increased DNA/nucleus during regeneration would not appear to be compatible with the views of Pasteels & Lison (1950*b*, *c*).

Our observation that the average DNAP content/nucleus was higher on the second and fourth days after operation than on the other days would suggest that the mitotic rate reached peaks on the second and fourth days. Although we are inclined to attach importance to these changes observed

from day to day during the first 4 days after hepatectomy, more experimental data will be needed to evaluate their significance. Price & Laird (1950), using a different method of estimation, found that the average DNA/nucleus fell steadily from 18·2 pg. (equivalent to 1·77 pg. DNAP) 24 hr. after operation to 11·8 pg. (equivalent to 1·15 pg. DNAP) at 96 hr., and then remained fairly constant till the eighth day. In our experiments, and also in those of Price & Laird (1950), the average DNA content/nucleus was still above the normal level 6–10 days after operation, when the phase of rapid growth had ended. This may be due to a higher proportion of polyploid nuclei, since Beams & King (1942) and Sulkin (1943) found that the proportion of polyploid nuclei in the parenchymal cells is increased after partial hepatectomy. In this respect the difference between the normal and restored livers would be analogous to the difference between the livers of the young and the adult animals for, in both cases, cell multiplication leads to an increase in the proportion of polyploid nuclei. According to Beams & King (1942) these are formed during mitosis when the chromosomes from both nuclei of a binucleate cell unite on a common metaphase plate.

In summary, in the resting nucleus each set of chromosomes probably contains a constant amount of DNA. Most somatic tissues are diploid and their nuclei ordinarily have an amount of DNA corresponding to two sets of chromosomes. Some organs, such as the rat liver, normally have a greater average amount of DNA/nucleus due to the presence of polyploid cells. Before the onset of mitosis there is an increase in the DNA content of the nucleus, with the result that tissues that are growing very actively have a greater-than-normal average DNA content/nucleus, due to the presence of nuclei that are about to undergo mitosis.

In adult tissues in which mitoses are infrequent the average DNA content/nucleus should not be affected by any circumstance or treatment that does not lead to either multiplication or destruction of the nuclei. The results shown in Table 2, section 2, support this conclusion. None of the dietary conditions affected the average DNA content of the liver nuclei, although some of them produced marked changes in the chemical composition or histological appearance of the liver. Similar results for the effect of fasting have been reported by Mirsky & Ris (1951) and McIndoe & Davidson (1952), and for the effect of protein deficiency by Campbell & Kosterlitz (1952).

The response of young animals to protein deficiency may differ from that of adults. Ely & Ross (1951*a*, *b*) reported that in rats weighing 130–150 g. the average DNA content/nucleus in the liver, pancreas and small intestine was higher in animals maintained on a protein-free diet for 8–49 days than in animals maintained on a complete diet. Lecomte & Smul (1952) found by cytochemical estimations on individual nuclei that in rats weighing 60–65 g. protein deficiency for 28 days resulted in an increase in the DNA content of the liver nuclei. We did not attempt similar experiments because it is very difficult to evaluate the results. Many, possibly most, of the cells in the livers of young growing animals will eventually undergo mitosis. Before doing so they will double their content of DNA. It is conceivable that protein deficiency might inhibit growth and mitosis to a greater extent than it affects the premitotic synthesis of DNA, and this could result in an apparent increase in the average DNA content per nucleus.

The results for the composition of adult whole liver (Table 3) confirm and extend the observations made by Campbell & Kosterlitz (1950), by Mandel (1951) and by Davidson (1947, 1952) on the remarkable constancy of the total DNA content of the liver, i.e. of the total number of cells that it contains. In this organ the nuclei and their characteristic chemical component, DNA, form a constant element while LP, PN and cell mass (in short, the cytoplasm) vary a great deal, depending on the nutritional status of the animal. RNA follows an intermediate pattern; in the initial stages of conditions like starvation or protein deficiency there is a marked decrease in the RNA content of the liver, but this trend does not seem to be progressive like the changes in the constituents mentioned above. A similar trend can be seen in the data of Kosterlitz (1947). Such behaviour suggests that only part of the RNA in the cell is 'labile' and it might be related to the observations made by Muntwyler and his associates who found that protein deficiency had affected the RNA content of the supernatant fraction much less than that of other cellular fractions isolated from homogenized livers by differential centrifugation (Muntwyler, Seifter & Harkness, 1950; Seifter, Muntwyler & Harkness, 1950). The effect is more clearly seen if the data are re-calculated on the basis of total liver contents. Munro, Wikramanayake & Heagy (1952) have also observed that protein deficiency has a selective effect on the RNA content of different cellular fractions.

An important practical consequence of the constancy of the average DNA content is that it provides a fairly simple method of obtaining an estimate of the number of cells in a tissue for calculations of the average composition/cell. It is necessary to relate the results of tissue analysis to some characteristic that has been selected as a standard—most frequently wet or dry weight. This has the grave disadvantage that an apparent

change in the measured variable in fact may be due to a change in the standard or reference. For example, during a 48 hr. fast the concentrations of DNAP, LP and RNAP in terms of unit wt. of liver tissue increased (Table 3, section 4). But calculations of the total amounts/liver show that the DNAP content remained unchanged while the LP, PN and RNAP actually decreased by 20–30%. The apparent increase in these constituents, on a wet wt. basis, was due to a reduction of almost 40% in the liver wt. and could be quite misleading. Obviously, it is desirable that the standard of references should not change during the experiment.

The simplest solution of the problem is to quote the total amount of each component/liver, as is done in the example above. This has two limitations; it is only applicable when a whole organ is available for analysis and cannot be used for tissues such as bone marrow or biopsy specimens; changes in cell number cannot be distinguished from changes in average cell composition. A better procedure is to use the DNA content of the tissue as a measure of cell number and to use the ratios of other constituents to the DNA as measures of average cell composition. This overcomes the limitations of the first method and has the additional advantage that it may bring to light significant changes in cell composition that otherwise would be obscured by random variation in cell number. For example, the changes in the liver wt. and total content of LP and RNAP during the second week on the protein-free diet (Table 3, section 4) are not statistically significant while the change in total PN content is significant only on the 5% level. If the results are referred to DNAP, however, it becomes apparent that there are significant decreases in average cell mass ($P < 0.01$) and average cell content of LP ($P < 0.01$) and PN ($P < 0.02$) while the average RNAP content/cell has not changed.

We believe that when analytical results are expressed in relation to DNA content they suggest a more accurate interpretation than when presented in conventional form. It should be emphasized, however, that if the experiment results in a change in the average DNA content/nucleus this change must be taken into account in interpreting the results in order to visualize changes in the average composition/cell.

In our experiments on adult animals the average DNA content/nucleus changed in the hepatectomy experiment, which has already been discussed, and in the long-term experiment with a carcinogenic diet (Table 4) in which the average DNAP content/nucleus in the liver was much lower than the usual adult figure. In a tumour-bearing tissue at least three distinct processes might affect the average cell composition. (1) As the number of tumour cells increases the average cell composition for the tissue will approach the average composition of the tumour cells. If the tumour cells are smaller than the normal cells, the average cell mass for the whole organ will fall. If the tumour cells have diploid nuclei the average DNA content/nucleus will fall in an organ like the adult rat liver which normally contains polyploid nuclei (Table 4). If the tumour nuclei contain more DNA than the nuclei of the normal tissue, as happens in at least one fowl tumour (McIndoe & Davidson, 1952), the average will be higher in the cancerous tissue. (2) If the tumour is growing at a fairly rapid rate it may contain a significant proportion of nuclei that are about to divide and have more than the normal resting complement of DNA. This would tend to raise the average DNA content/nucleus. (3) If the growing nodules of tumour tissue compress the normal tissue to such an extent that it degenerates, the nuclei of the latter may undergo pyknosis and lose DNA (Leuchtenberger, 1950). This will reduce the average DNA content/nucleus. Of the three processes, the first usually would be much the most important quantitatively.

SUMMARY

1. The average deoxyribonucleic acid phosphorus (DNAP) content/nucleus has been determined in various tissues of the rat by chemical analysis of isolated nuclei. For the liver the DNAP, ribonucleic acid phosphorus (RNAP), lipid phosphorus (LP) and protein nitrogen (PN) contents of the whole organ were estimated and the effects of such factors as sex, age, strain and diet were investigated. The average cell composition in the liver was calculated from the average DNAP content of its nuclei and the results of the whole tissue analysis.

2. In spleen, kidney, pancreas, small intestine, leucocytes, bone marrow, thymus, heart, lung and salivary gland the average DNAP content/nucleus is of the order of 0·65–0·70 pg. in both young and adult animals.

3. In the liver the average DNAP content/nucleus is of the order of 0·90 pg. in adult rats and 0·75–0·80 pg. in embryos and young animals.

4. In the adult rat the average DNAP content/nucleus in the liver does not vary with sex, strain or body wt. and is not affected by fasting, by a protein-free diet, by a thiamine-deficient diet, by a high-fat diet, by thioacetamide, by a diabetogenic dose of alloxan or by pregnancy.

5. The average DNAP content/nucleus is significantly decreased in the livers of adult rats following prolonged feeding of *p*-dimethylaminoazobenzene (DAB).

6. During the first 4 days following partial hepatectomy the average DNAP content/nucleus in the rat liver is 10–50% above the normal value.

7. The average cell mass and the average content/cell of RNAP, LP and PN in the liver are 20–30% lower in the normal female adult rat than in the male. In pregnancy the average cell mass and content/cell of RNAP, LP and PN are increased.

8. Fasting, protein deficiency and thiamine deficiency do not affect the number of cells in the liver but cause a decrease in the average mass and the RNAP, LP and PN content of its cells.

9. A diabetogenic dose of alloxan causes a decrease in liver wt. and in the total content of RNAP, LP and PN in the liver.

10. Thioacetamide has no effect on the composition of whole liver tissue, although it causes an increase in the size of the nuclei.

11. Prolonged administration of DAB results in an increase in the number of cells in the liver and a reduction of the average cell mass and average content of RNAP, LP and PN. These effects occur both in livers with obvious tumours and in livers that have not reached this stage.

12. The advantages of expressing results of tissue analyses in relation to DNA content are discussed.

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The Isolation of L-Pipecolinic Acid from *Trifolium repens*

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The isolation of L-pipecolinic acid (piperidine-2-carboxylic acid) from leaves of *Trifolium repens* (white clover) has already been briefly reported (Morrison, 1952), and the present paper gives a detailed account of the isolation and identification of the substance.

During the examination by paper chromatography of the amino-acids extracted by aqueous ethanol from the dried foliage of *T. repens*, a distinctive unidentified ninhydrin-reacting substance was observed. This substance had an R_F value in most solvents somewhat higher than that of proline, and produced with ninhydrin a characteristic deep violet-blue colour which faded very rapidly: Fig. 1 shows its position relative to the other amino-acids on a two-dimensional chromatogram of an ethanol extract of clover leaves. Unidentified spots, apparently similar, on paper chromatograms have been reported by Fowden (1951) from algae, by Campbell, Work & Mellanby (1951) from agenized wheat flour, and by Biserte & Scriban (1950, 1951) from malt and barley.

The substance was present in amount comparable with that of other amino-acids, and it seemed worthwhile to attempt to isolate and identify it. It was found possible to achieve a partial separation of the unknown from the other amino-acids by displacement chromatography on a column of a cation-exchange resin (Amberlite IR-100) after the method of Partridge & Westall (1949), and ultimately it was separated completely by partition chromatography on columns of powdered cellulose using a mixture of *n*-butanol with aqueous acetic or formic acid as solvent, and obtained in crystalline form. The progress of the separation was followed at each stage by means of paper chromatograms of the various fractions.

Identification. From its properties it appeared that the substance might be either pipecolinic acid (piperidine-2-carboxylic acid) or nipecotinic acid (piperidine-3-carboxylic acid). The described properties of optically active pipecolinic acid (Mende, 1896; Willstätter, 1901; Leithe, 1932) resembled closely those of the unknown substance. Nipecotinic acid has been described only in the racemic form (Ladenburg, 1892; Besthorn, 1895; Freudenberg, 1918), but its properties are very similar to

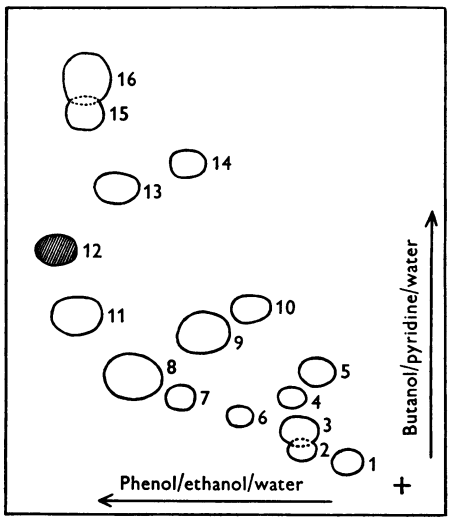

Fig. 1. Diagram of two-dimensional paper chromatogram of amino-acids in ethanol extract of leaves of *Trifolium repens* showing position of pipecolinic acid. 1, Aspartic acid; 2, glutamic acid; 3, asparagine; 4, glycine; 5, serine; 6, glutamine; 7, β-alanine; 8, γ-amino-*n*-butyric acid; 9, alanine; 10, threonine; 11, proline; 12, pipecolinic acid; 13, valine; 14, tyrosine; 15, phenylalanine; 16, leucine.