2. The nucleic acid is free from protein and from desoxypentose. It resembles yeast ribonucleic acid in its pentose content, in its absorption spectrum, in its action as a substrate for ribonuclease and in its content of purine and easily hydrolyzable phosphorus. Among the hydrolysis products *d*-ribose has been identified as the *p*-bromophenylhydrazine.

We should like to thank Dr Neil Campbell of Edinburgh University for supplying us with a specimen of pure *p*-bromophenylhydrazine, and for his advice on the preparation of hydrazones. This work was carried out during the tenure of a Carnegie Teaching Fellowship by one of us (J.N.D.). A grant for scientific assistance from the Medical Research Council and an expenses grant from the Carnegie Trust for the Universities of Scotland to one of us (J.N.D.) are gratefully acknowledged.

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Tissue Nucleic Acids

3. THE NUCLEIC ACID AND NUCLEOTIDE CONTENT OF LIVER TISSUE

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(Received 2 August 1944)

It has been shown previously (Davidson & Waymouth, 1944a, d) that ribonucleic acids are widespread in animal tissues and are present in high concentrations in rapidly growing embryonic tissues, although the proportion of ribonucleic acid to desoxyribonucleic acid appears in most cases to be similar in the embryo and the corresponding adult tissue. A ribonucleic acid has been isolated from the liver of the sheep and its properties described (Davidson & Waymouth, 1944b). It has been demonstrated histochemically in the cytoplasm of the

liver cell (Davidson & Waymouth, 1944c). It is of some interest, therefore, to determine the amount of this nucleic acid present in liver tissue and its possible variation under different circumstances. For such an examination liver tissue has many advantages. It is possible to compare normal adult liver not only with the embryonic organ but with the regenerating organ and with liver tumours produced by the administration of such agents as dimethylaminoazobenzene (butter yellow). This fact has already been utilized by Burk (1942) in an

examination of the glycolysis mechanisms in liver in different states. He was able to show that only in malignant liver tissue was anaerobic glycolysis markedly increased (see also Dickens & Weil-Malherbe, 1943). In regenerating liver it was normal, and the high values obtained for embryonic liver could be to a large extent accounted for by the amount of erythropoietic tissue present at this stage (Burk, 1942; Norris, Blanchard & Povolny, 1942). When the nucleic acid contents of embryonic and adult livers are compared, this difference in cellular composition must be recognized.

Higher nucleic acid contents have been found in embryonic sheep and fowl livers than in the adult tissues (Davidson & Waymouth, 1944*a*) and in newborn lamb than in adult sheep (Robertson & Dawbarn, 1929), and a fall in liver nucleic acid has been noted during embryonic development in the rabbit (Masing, 1911) and in the rat (Dumm, 1943).

Variations in the total nucleic acid contents of adult livers have also been observed with the age and nutritional state of the animal (Rondoni, 1941). A rise in nucleic acid concentration on fasting has been observed in the livers of many species (Kossel, 1882; Tichmeneff, 1914; Cahn & Bonot, 1928; Rosenthal & Drabkin, 1943; Kosterlitz & Cramb, 1943; Kosterlitz, 1944*a, b*), though this is accompanied by a fall in liver weight, due to decrease in cell volume, so that there is in fact a fall in liver nucleic acid (as well as in phospholipin and in protein) relative to the original body weight (Kosterlitz & Cramb, 1943).

When the median and left lateral lobes (65–75% of the whole organ) of the liver of the rat are removed, the remaining lobes undergo a process of rapid growth until in 28 days the liver has been restored to its original weight (Higgins & Anderson, 1931). This growth process is usually termed 'regeneration', although Sulkin (1943) points out that it would more correctly be designated a compensatory hyperplasia. The most active phase occurs about the third day after operation when mitotic figures are most numerous (Burk, 1942; Norris, Blanchard & Povolny, 1942). During the first 24 hr. after partial hepatectomy the mass of the residual liver fragment increases markedly by increase in cell size without cell division (Brues, Drury & Brues, 1936). During the process of regeneration the nucleic acid metabolism of the liver is profoundly affected. Using P^{32} , Brues, Tracy & Cohn (1942) have shown that while in normal rat liver the (desoxyribo)nucleic acid P has a lower turnover rate than the non-(desoxyribo)nucleic acid protein-bound P, in the regenerating liver the turnover rate of the nucleic acid P is increased up to the level of the non-(desoxyribo)nucleic acid protein-bound P.

The liver is, moreover, an organ in which experimental tumours can be produced without great

difficulty by feeding the dyestuff 'butter yellow', *p*-dimethylaminoazobenzene (cf. Sugiura & Rhoads, 1941). The process involves a 'proliferation of connective tissue cells in the portal systems with degeneration of the contiguous liver cells, a typical regenerative proliferation of bile duct and liver epithelium leading ultimately to non-architectural nodular hyperplasia and a macroscopically hob-nailed liver' in which three types of tumour may arise, liver-cell carcinoma, bile-duct carcinoma and bile-duct cystadenoma (Orr, 1940). Liver is indeed one of the few tissues in which it is possible to make a direct comparison between tumour tissue and its tissue of origin (Dickens & Weil-Malherbe, 1943). Tumour formation is associated with a rise in nucleic acid content (Fujiwara, Nakahara & Kishi, 1937; Dickens & Weil-Malherbe, 1943).

With rat liver, therefore, it is possible to compare normal adult liver tissue with rapidly growing liver tissue in the case of the embryonic or 'regenerating' organ and with the cirrhotic or tumour-bearing organ.

METHODS

Animals. All experiments were carried out on adult hooded rats supplied by the Rowett Institute and fed, unless otherwise stated, on the stock diet described by Thomson (1936).

Pregnant animals were used 1–2 days before parturition was expected, in order that embryos of reasonable size might be obtained. The embryos were removed from the uterus, quickly weighed, and the livers excised, pooled and dealt with as for adult liver.

In the experiments involving regenerating liver, rats under 'Nembutal' anaesthesia were partially hepatectomized by the method of Higgins & Anderson (1931). For the first 16 hr. after operation the animals received only a solution of glucose in water. Subsequently they received stock diet. In a control group of animals the abdomen was opened under 'Nembutal' anaesthesia and closed again without removal of hepatic tissue. The rats were killed on the third day after operation, after a 24 hr. fast.

For the experiments on hepatic tumours three groups of rats were chosen. Two groups were fed on husked but unpolished rice, to each kg. of which were added 25 ml. of a 3% solution of dimethylaminoazobenzene (A.R.—British Drug Houses Ltd.) in arachis oil. Each rat received a slice of raw carrot daily and water *ad lib.*, and was starved for 24 hr. before use. One group was used after 100 days and another after 150 days. The third group of rats received rice with arachis oil but without dye, and served as controls. Their livers were examined at the end of 100 days.

Estimation of nucleic acids, etc. The animals were killed by decapitation and the livers immediately excised and divided into portions thus: One portion was ground in ice-cold trichloroacetic acid for estimation of nucleotide and nucleoside by the method of Kerr (1940). A second portion was dried in a tared beaker at 100° to constant weight for determination of water content. A third portion was fixed for histological examination. A fourth small portion was used for determination of total nucleoprotein phosphorus (NPP) or residual P by the method of Berenblum, Chain & Heatley (1939). The remainder of the tissue was used for

the estimation of the relative amounts of the two types of nucleic acid.

The tissue was minced, dehydrated with several successive portions of ethanol and of ether, and dried. The dried material was transferred to a stoppered 50 ml. centrifuge tube and shaken for four 1 hr. periods with successive portions of about 40 ml. 0.1N-HCl. This process removes acid-soluble P, including simple nucleotides. The residue was washed twice with ethanol and was then extracted for two successive 2 hr. periods at 65° under a reflux condenser with an ethanol-chloroform mixture (3:1). It was then washed with ether, dried, ground to a fine powder in a mechanical mortar and passed through a sieve (120 mesh). In cases where the amount of powder available from one liver was small, material from several livers was pooled for analysis. Total P was estimated in the extracted powder by a modification of the method of Allen (1940).

Ribonucleic acid phosphorus and deoxyribonucleic acid phosphorus. The relative amounts of ribonucleic acid phosphorus (RNAP) and deoxyribonucleic acid phosphorus (DNAP) in the extracted powder were determined by two different methods. One involved the extraction of the nucleic acids with 10% NaCl, their precipitation as lanthanum salts, and analysis of the precipitate for pentose and desoxypentose by modifications of methods previously described (Davidson & Waymouth, 1944a). As this procedure in the case of liver tissue involved certain losses which were relatively greater for deoxyribonucleic acid than for ribonucleic acid, direct estimations of pentose and desoxypentose were also undertaken on the extracted tissue powders. Such estimations yield useful information in the case of liver tissue though it is doubtful if they could be applied to other tissues indiscriminately.

(a) *Direct method.* Portions of powder expected to contain 100–200 µg. RNAP were used for pentose, and 100–300 µg. DNAP for desoxypentose, estimations.

Pentose estimation. Appropriate portions of powder (usually 20 mg.) were weighed into each of two graduated 10 ml. centrifuge tubes. To one was added 2 ml. water and 2 ml. Meibbaum's (1939) reagent (100 mg. orcinol in 10 ml. conc. HCl containing 0.1% FeCl₃·6H₂O), and to the other 2 ml. water and 2 ml. of HCl containing 0.1% FeCl₃·6H₂O but no orcinol. Both tubes were heated with stirring in a boiling water-bath for exactly 10 min. and cooled. The blue-green material was extracted successively with 2, 2 and 1.5 ml. amyl alcohol. The amyl-alcohol layers were separated by centrifuging, combined, mixed with 1 ml. ethanol, and made up to a total volume of 8 ml. with amyl alcohol. Exactly similar extractions were carried out on the blank tube (without orcinol). The coloured solution was read against the blank in the Hilger Spekker absorptiometer, with the Blue (no. 7) colour filter. A calibration curve was prepared from yeast ribonucleic acid, for which 2 ml. portions at various concentrations (25–200 µg. P) were treated as in the estimation.

Estimation of desoxypentose was carried out by a modification of the diphenylamine reaction employed by Sevag, Smolens & Lackman (1940) and Dische (1930). An appropriate amount of powder (usually 200 mg.) was heated in a graduated 10 ml. centrifuge tube with 8 ml. 0.1N-HCl for 20 min., with frequent stirring. After cooling, the volume was made up to 8 ml. and the mixture was centrifuged. A 3 ml. portion was heated for exactly 6 min. in a briskly boiling water-bath with 8 ml. diphenylamine reagent (1 g. A.R. diphenylamine, twice recrystallized from ethanol, 2 ml. conc. H₂SO₄ and 98 ml. A.R. glacial acetic acid).

A blank was prepared from a second 3 ml. of the supernatant fluid and 8 ml. acetic acid. The cooled solution was read against the blank in the Hilger Spekker absorptiometer, with the Red (no. 1) colour filter. A calibration curve was prepared from deoxyribonucleic acid, 4 ml. portions being hydrolyzed in HCl of final concentration 0.1N, 3 ml. of the supernatant being used for the development of the colour with 8 ml. of reagent.

(b) *Method for lanthanum salts.* The nucleic acids were extracted from the tissue powders with 10% NaCl and precipitated as lanthanum salts. The solution of nucleic acids recovered from the lanthanum precipitate was then used for the estimation of pentose and desoxypentose by a modification of the method previously employed (Davidson & Waymouth, 1944a).

For the nucleic acid extraction, a suitable quantity of tissue powder (e.g. 400 mg.) was weighed into a 15 ml. conical centrifuge tube and 4 drops triacetin added. Extraction with 10% NaCl was carried out with five successive portions: (a) 3 ml. overnight at 0°, (b) 2 ml. for 30 min. at 100°, (c) 2 ml. for 30 min. at 100°, (d) 2 ml. for 10 min. at 100°, and (e) 2 ml. for 10 min. at 100°, respectively.

The extracts were combined and made up to 10 ml. 0.5 ml. was taken for total P estimation. 9 ml. were pipetted into a 25 ml. centrifuge tube and 1 ml. 2% lanthanum acetate and 10 ml. ethanol added. After 1 hr. at 0° the precipitate was centrifuged down and washed twice with 3 ml. 0.2% lanthanum acetate. The moist precipitate was decomposed with two successive 0.25 ml. portions of 0.5M-Na₂CO₃. The lanthanum carbonate was centrifuged down and the extract made up to 5 ml.

The extract was treated as follows: (i) 0.5 ml. was taken for total P estimation. (ii) 0.2–0.3 ml. was pipetted into a pyrex tube for estimation of pentose by a modification of the method of Meibbaum (1939). The volume was made up to 2 ml. with water and 2 ml. Meibbaum's reagent (*vide supra*) were added. The tube was heated in a boiling water-bath for 30 min. as recommended by Schlenk (1942) and was then cooled. 2 ml. ethanol and 4 ml. water were added and the green colour read off in the Hilger Spekker absorptiometer (Filter no. 7) with a calibration curve drawn up in terms of RNAP and made from liver ribonucleic acid precipitated as lanthanum salt and treated as described above. (iii) 3 ml. were taken for the colorimetric estimation of desoxypentose by the diphenylamine reaction, and were pipetted into a conical graduated centrifuge tube containing 1 ml. 0.55N-HCl. The tube was heated in a boiling water-bath for 20 min., cooled, and the volume made up to 4 ml. 8 ml. freshly made diphenylamine reagent (*vide supra*) were added and the tube was placed in the briskly boiling water-bath for 6 min., quickly cooled, and the blue colour read off after 5 min. in the Hilger Spekker absorptiometer (Filter no. 1), with a calibration curve drawn up in terms of deoxyribonucleic acid phosphorus (DNAP) and made from thymus deoxyribonucleic acid precipitated as lanthanum salt.

Neither the total P of the extracted powder nor the ratio RNAP/DNAP was appreciably altered when the fresh livers were allowed to stand overnight at room temperature.

RESULTS

The results of the nucleoprotein phosphorus (NPP) and moisture estimations are shown in Table 1. The moisture content in most cases remains constant at about 70% but is higher in embryo liver and in liver

Table 1. *Water and nucleoprotein phosphorus (NPP) content of rat livers*

Mean values and the standard error are shown in each case. The figures in brackets are the numbers of animals employed.

Type of liver tissue	Water content (%)	Nucleoprotein phosphorus (NPP) (mg./100 g.)		Weight of rats (g.)	Weight of livers (g.)	Weight of liver as % body wt.
		Wet wt.	Dry wt.			
Normal male (fed)	69.9 ± 0.412 (9)	134 ± 2.56 (14)	449 ± 7.33 (14)	361 ± 16.09 (9)	10.6 ± 0.55 (6)	2.89 ± 0.10 (6)
Normal male (fasted 24 hr.)	69.7 ± 0.195 (11)	152 ± 2.65 (16)	502 ± 9.84 (16)	316 ± 22.83 (8)	7.9 ± 0.54 (8)	2.51 ± 0.10 (8)
Normal female (fed)	70.0 ± 0.263 (12)	137 ± 3.74 (12)	452 ± 13.25 (12)	172 ± 5.49 (11)	7.1 ± 0.36 (7)	4.17 ± 0.15 (7)
Normal female (fasted 24 hr.)	69.9 ± 0.237 (12)	142 ± 3.66 (12)	473 ± 12.81 (12)	168 ± 6.80 (10)	5.1 ± 0.22 (6)	3.39 ± 0.13 (6)
Pregnant female (fasted 24 hr.)	71.0 ± 0.258 (6)	156 ± 3.64 (6)	543 ± 12.06 (6)	—	—	—
Embryo	80.6 ± 0.271 (8)	174 ± 13.27 (8)	908 ± 101.5 (8)	2.6 ± 0.24 (8)	—	—
Regenerating (male)	70.0 ± 0.395 (9)	153 ± 3.18 (9)	512 ± 12.04 (9)	350 ± 15.12 (8)	7.3 ± 0.32* (8)	—
Laparotomy (male)	69.5 ± 0.229 (6)	150 ± 1.64 (8)	490 ± 5.47 (8)	353 ± 12.04 (6)	9.0 ± 0.40 (7)	—
Control diet (male)	70.6 ± 0.111 (6)	149 ± 2.44 (6)	505 ± 6.97 (6)	224 ± 3.05 (6)	—	—
Liver tumours (100 days) (male)	73.8 ± 0.444 (6)	143 ± 1.64 (6)	543 ± 10.95 (6)	179 ± 6.27 (6)	—	—
Liver tumours (150 days) (male)	76.0 ± 0.731 (5)	128 ± 6.54 (5)	535 ± 40.74 (5)	163 ± 4.95 (4)	—	—

* The mean weight of liver tissue removed 3 days previously at partial hepatectomy was 6.8 ± 0.46 g.

tumour. The high moisture content of the rapidly growing tissues of embryos and tumours has already been commented on (Davidson & Waymouth, 1944a). The NPP figures for the rat liver are slightly higher than those quoted by other authors (Rondoni, 1941; Berenblum, Chain & Heatley, 1939; Kosterlitz & Cramb, 1943) and are closer to those of Fujiwara, Nakahara & Kishi (1937). The NPP rises on fasting. In our series of normal male rats (340 ± 60 g.) the difference between the NPP of livers of fed and starved animals is statistically significant. In the series of female rats (170 ± 30 g.) it is not significant but the difference between the total P content of the extracted powders from the livers of fed and starved female rats is significant. The weight of the liver as a percentage of the body weight shows a significant fall in the starved animal (male or female). The liver of a pregnant rat has a particularly high NPP content but the highest values are found in the embryo. The weights of the embryos used varied from 3.4 to 1.5 g. and the NPP values from 125 to 246 mg./100 g. wet weight or 596 to 1440 mg./100 g. dry weight.

The regenerating livers show slightly higher NPP values than those of the controls, but the difference is not statistically significant. On the other hand, the difference between the values for the 100-day liver tumours and those of the controls is significant.

The NPP of the tumours is lower, on a wet-weight basis, than that of the livers of rats on the control diet, but on a dry-weight basis it is higher, on account of the high water content of the tumours.

Results of the nucleotide, etc. estimations are shown in Table 2. There is no significant difference in nucleotide concentration or in total soluble purine concentration in the livers of fed and fasted rats. This confirms the tentative conclusion arrived at by Rapoport, Lever & Guest (1943). Embryo liver, like other embryonic tissues, has a particularly low nucleotide concentration. The nucleotide and total purine concentration in regenerating livers is significantly higher than that of controls. In the 100-day tumours the nucleotide concentration is significantly lower on a wet-weight, but not on a dry-weight, basis than that of the controls. No appreciable variations in the nucleoside + free purine fraction were found.

The results of the analyses of the extracted tissue powders are shown in Table 3. The total P content runs parallel with the NPP figures but is expressed here in terms of dry fat-free material. In most cases more than 90 % of this total P can be accounted for in terms of ribonucleic acid phosphorus (RNAP) and desoxyribonucleic acid phosphorus (DNAP). The powders from livers of starved animals show a lower RNAP content and a higher DNAP content

Table 2. *Nucleotide N, nucleoside N, and free purine N contents of rat livers*

The mean and the standard error are shown in each case. The number of animals is given in brackets.

Type of liver tissue	Nitrogen content* (mg./100 g.)		
	Nucleotide N	Nucleoside N + free purine N	Total acid-soluble purine N
Normal male (fed)	30.6 ± 1.01 (6)	9.7 ± 1.20 (6)	40.3 ± 0.62 (6)
Normal male (fasted 24 hr.)	31.1 ± 0.68 (10)	10.2 ± 0.74 (9)	41.4 ± 0.83 (9)
Embryo	17.1 ± 0.89 (6)	7.3 ± 0.30 (6)	24.4 ± 0.79 (6)
Regenerating	36.1 ± 1.46 (8)	10.9 ± 1.41 (8)	47.0 ± 1.87 (8)
Laparotomy	32.0 ± 1.09 (8)	9.9 ± 0.59 (7)	41.1 ± 1.18 (7)
Control diet	31.4 ± 1.48 (6)	9.9 ± 0.71 (6)	44.0 ± 1.23 (6)
Tumours (100 days)	28.8 ± 0.28 (6)	10.7 ± 0.67 (6)	39.6 ± 0.92 (6)
Tumours (150 days)	17.3 ± 3.31 (5)	9.9 ± 1.24 (5)	27.3 ± 3.43 (5)

* The sum of nucleotide N + nucleoside N + free purine N gives the total acid-soluble purine N content.

Table 3. *Ribonucleic acid phosphorus (RNAP) and deoxyribonucleic acid phosphorus (DNAP) content of extracted liver powders, as estimated directly and on the precipitate of lanthanum salts*

The figure in brackets shows the number of powders examined. Each powder represents, in most cases, pooled material from several animals.

Type of liver tissue	Total P in extracted powder (mg./100 g.)	RNAP as % total P		DNAP as % total P		RNAP + DNAP as % total P		Ratio: RNAP/DNAP	
		Direct	From lanthanum ppt.	Direct	From lanthanum ppt.	Direct	From lanthanum ppt.	Direct	From lanthanum ppt.
Fed	554 ± 10.72 (10)	75.3 ± 2.33 (10)	81.9 ± 1.89 (10)	17.6 ± 0.59 (10)	10.6 ± 0.51 (10)	92.6	93.5	4.3	8.1
Fasted	583 ± 20.46 (9)	66.0 ± 2.86 (9)	75.8 ± 1.82 (9)	20.2 ± 0.84 (9)	13.3 ± 0.52 (9)	86.7	89.1	3.1	5.8
Pregnant	774 (1)	75	77	17.7	10	93	86	4.2	7.8
Regenerating	690 (2)	72	90	18	8.5	90	98	4.0	10.9
Laparotomy	603 (2)	85	88	22.2	10.5	107	97	3.8	8.5
Control diet	716 (1)	61	85	13.7	13	75	98	4.5	6.5
Tumours	738 (2)	71	75	19.5	18.9	90.5	94	3.6	4.0
Embryo	1720 (1)	49.5	70	33.7	28	83.5	98	1.5	2.5

than do those of fed rats, and both these differences are statistically significant. The ratio RNAP/DNAP is therefore reduced by fasting. This ratio is almost the same in the regenerating as in the control livers. In the tumours it is lower than in the corresponding controls, but the actual amounts of RNAP and DNAP determined in the tumour powders are both higher than those in the controls. Owing to the high total P content of livers of pregnant rats the total RNAP content is particularly high. In embryonic liver the ratio is low, and the extracted powder contains a higher RNAP content and DNAP content than does any of the other liver powders, but the relative amount of DNAP is higher. This is due in part at least to the presence of erythropoietic cells in the embryo liver. A lower RNAP/DNAP ratio in the embryo liver than in the adult liver has already been noted in the case of the sheep (Davidson & Waymouth, 1944a).

The ratios of RNAP/DNAP obtained for various forms of rat liver tissue indicate that a large proportion (some 80 %) of the total nucleic acid of rat liver tissue is present in the form of ribonucleic acid. Similar ratios have been obtained with the liver tissue of other species (sheep, pig, horse, man).

The ratios obtained from the analysis of the precipitate of lanthanum salts are in most cases considerably higher than those found on direct analysis of the liver powder. Owing to losses in extraction of the nucleic acids, in precipitation as lanthanum salts and on resolution from the lanthanum precipitate, the amount of P in the material recovered from the lanthanum precipitate is, in the case of liver tissue, only about 60 % of that in the tissue powder. These losses are proportionately greater for desoxyribo- than for ribonucleic acid, and the RNAP/DNAP ratios are therefore high. Nevertheless the ratios obtained for various forms of liver show the same relative variations among themselves as do the ratios obtained by direct analysis.

DISCUSSION

The rise in NPP concentration in liver tissue found in fasting animals confirms the earlier observations of other workers (Kossel, 1882; Tichmeneff, 1914; Cahn & Bonot, 1928; Rosenthal & Drabkin, 1943; Kosterlitz & Cramb, 1943). Kosterlitz (1944a, b) has shown that rats starved or placed on a protein-poor diet show a loss in stainable material and of

mitochondria in the cytoplasm of the liver cell. The particulate components of the cytoplasm are reported by Claude (1943*a, b*), Bensley (1942), Hoerr (1943) and Lazarow (1943) to contain a nucleic acid of the pentose type which is presumably identical with the ribonucleic acid which we have isolated from liver tissue (Davidson & Waymouth, 1944*b*). The fall which we have observed in the RNAP/DNAP ratio in fasting rats would be consistent therefore with a diminution in the phospholipin-ribonucleoprotein complex content of the liver cytoplasm. The loss on fasting of liver weight as a percentage of body weight is in agreement with the results of other workers (e.g. Kosterlitz & Cramb, 1943).

Comparison of normal adult liver tissue with rapidly growing liver tissue from the embryo is rendered difficult by the peculiar cellular content of the latter but such a comparison can be made between normal and regenerating liver. The livers of rats submitted to laparotomy give figures for nucleic acid and nucleotide content which are very close to those obtained for normal male starved rats. The regenerating livers differ appreciably only in one respect, their high simple nucleotide and total soluble purine concentration. In this they differ from other rapidly growing tissues (embryonic and neoplastic), which have characteristically low acid-soluble nucleotide concentrations (Davidson & Waymouth, 1944*a, d*). Such low concentrations are found in the embryonic rat liver and in the liver tumours.

The NPP of the 100-day liver tumours is higher than that of the control livers when the comparison is made on a dry-weight basis, but the difference is due largely to the increased water content of the tumours. In no case was the difference between the NPP (dry-weight basis) of the liver tumours and that of normal rat liver as great as that recorded by Fujiwara, Nakahara & Kishi (1937). On a wet-weight basis the NPP figures are of the same order for liver tumours, livers from rats on the control diet and livers from normal fasting male rats. On the other hand, the P contents of the extracted liver powders are much higher in the case of both liver tumours and livers from rats on the control diet than in the case of livers of normal starved rats. The P content of the extracted powder from liver of pregnant rats is also at the same high level.

Caspersson, Nyström & Santesson (1941) and

Caspersson & Santesson (1942) have reported the presence of high concentrations of ribopolynucleotides in the cytoplasm of some tumour cells. Although the concentration of RNAP in the liver tumours is higher than that of the controls the difference is in no case very great. The DNAP concentration is also raised and the RNAP/DNAP ratio is lower than that of the controls.

It is now clear that liver tissue contains ribonucleic acid, that this acid is found in the cytoplasm of the liver cell, that it forms a large proportion of the total nucleic acid of liver tissue, and that it may vary independently of the nuclear desoxyribonucleic acid.

SUMMARY

1. The nucleic acid and nucleotide concentrations have been examined in a series of livers from fed and starved rats, from the pregnant and embryo rat, from the regenerating rat liver and in liver tumours induced by feeding butter yellow.

2. The total nucleic acid concentration, as measured by the nucleoprotein phosphorus, rises on fasting, is high in pregnancy and very high in the embryo. It is not appreciably altered in the regenerating liver, and is slightly raised in liver tumours. The acid-soluble nucleotide concentration is unaltered on fasting, is raised in regenerating livers, is low in liver tumours and very low in embryo livers.

3. Of the total nucleic acid a large proportion (70-90%) is present as ribonucleic acid. On fasting, the ribonucleic acid concentration falls by about 12% while the desoxyribonucleic acid concentration rises, both changes being statistically significant. The ratio of ribonucleic acid P to desoxyribonucleic acid P therefore falls on fasting, is the same in regenerating as in control livers, and is lower in liver tumours and in embryo livers than in the normal liver, although in both liver tumour and in the embryo, particularly the latter, the concentrations of both ribonucleic acid and desoxyribonucleic acid are higher than in normal liver.

This work was carried out during the tenure of a Carnegie Teaching Fellowship by one of us (J.N.D.). A grant for scientific assistance from the Medical Research Council and an expenses grant from the Carnegie Trust for the Universities of Scotland to one of us (J.N.D.) are gratefully acknowledged. We wish to thank Mr E. Alexander for valuable technical assistance.

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The Determination of Thiocyanate in Blood Serum

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(Received 31 July 1944)

Body water is distributed in three main compartments, blood plasma, cell fluid and the fluid in the intercellular spaces (interstitial fluid). In order to study changes in the distribution of water between these compartments it is necessary to be able to measure their volumes accurately. Crandall & Anderson (1934) have described a method for determining the volume of water available to thiocyanate. It has been inferred that this volume is equivalent to the interstitial fluid volume (Peters, 1942). Their method involves the injection of a known amount of sodium thiocyanate intravenously followed by the determination of its concentration in the blood serum after sufficient time has been allowed for its complete distribution.

The concentration of thiocyanate in the serum is determined by treating a trichloroacetic acid filtrate of serum with a solution of ferric nitrate; the orange colour which develops is measured colorimetrically. The standard is prepared by adding a known amount of thiocyanate to serum and treating it by the same process.

In this laboratory, attempts to follow changes in the volume of the interstitial fluid in cases of traumatic shock have met with little success. Determinations at intervals on the same patient gave inconsistent results. The technique of estimation was investigated, and it was found that when the colour was compared photoelectrically with that of an aqueous solution of NaSCN instead of a standard

filtrate, recoveries were about 7.5% low. This was due to the trichloroacetic acid interfering with the colour development in the filtrate. Attempts to obviate this error by the use of different protein precipitants (*p*-toluenesulphonic acid, uranyl nitrate, etc.) were unsuccessful; low recoveries were still obtained.

Conditions affecting the colour development were then studied. It was found that in Crandall & Anderson's method the concentration of the reagent is far below the optimum. The use of a modified method gave quantitative recoveries which were unaffected by the trichloroacetic acid.

EXPERIMENTAL

Colorimeter. A tungsten filament source was used, infrared radiation being removed by a 2 mm. thickness of Chance's 'Calorex' glass. The solution was measured in a 2 cm. solution thickness. A blue filter was used, Ilford 'Spectrum Blue' no. 602, with a maximum transmission at 4700 Å.

Standard thiocyanate solution. When 500 mg. NaSCN have been injected into an average-sized man, the concentration of thiocyanate in the serum is about 3 mg. NaSCN/100 ml. A solution of NaSCN slightly stronger than 0.1N was made by dissolving 11.5 g. of the salt in water and diluting to 1 l. This was standardized by titration against 0.1N-AgNO₃. The concentration was then adjusted to be exactly 0.1N-NaSCN, equivalent to 8.11 g. NaSCN/l. By diluting 35.1 ml. of the 0.1N-solution to 1 l., and again diluting 35.1 ml. of this solution to 1 l., a concentration of