CONCERNING AN ASPECT OF DESOXYRIBONUCLEIC ACID SYNTHESIS IN REGENERATING RAT LIVER

by

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The present state of our knowledge concerning the metabolism of adenine by the rat is included in a recent review by BROWN[1]. A very interesting facet of this work has been the demonstration that nucleic acid synthesis from adenine is limited to the ribonucleic acid fractions of tissue unless cell division is taking place. Indeed, in adult rat liver, the relative specific activities of ribonucleic acid (RNA) adenine and desoxyribonucleic acid (DNA) adenine were in the ratio of over 50:1, and this 24 hours following 3 days feeding at a dose level of 0.2 mmole/kg[2]. Adenine appears to be unique in this respect since all other carbon and nitrogen purine precursors show ratios of less than 10:1, (*e.g.* formate[3], glycine[4–7], serine[4], and cytidine[8]). Phosphate alone shows any similarity to adenine in that a ratio of 33:1 has been observed[9], but this was only 2 hours after a single injection of labelled sodium phosphate.

It was considered possible to utilise this effect by causing adult rat liver, extensively labelled in the RNA fraction by previous adenine injections, to undergo the sudden cell division which follows partial hepatectomy. Comparison of the specific activities of the DNA purines from the resting liver (removed at partial hepatectomy) with the DNA purines isolated from the regenerated liver should show whether the newly synthesised DNA had been formed from some labelled precursor in its environment.

METHODS

Radioactivity measurements of separated ribonucleotides and of purine bases were carried out as described previously[10]. White male rats were used throughout, fed a normal laboratory diet. The animals were given 6 intraperitoneal injections over 3 days of adenine-8-^{14}C hydrochloride[11] in water, at a level of about 0.2 mmole/Kg/day: the exact dose for each experiment is given in the Tables of results. The rats were starved for 24 hours following the last injection and, under ether anaesthesia, both central and the left lateral lobes of the liver removed[12]. The wound was stitched up and the animals allowed food and water freely during which time the acid-soluble fraction and the mixed nucleic acids were isolated from the liver removed (referred to hereafter as resting liver). 48 hours after the operation, the remaining liver, which had undergone compensatory hyperplasia ("regenerated liver"), was removed and worked up for acid-soluble fraction and mixed nucleic acids in exactly the same way as the resting liver.

Since the results were dependent on the measurement of the specific activities (counts/minute/μmole) of the DNA purines which had much lower activities than the corresponding RNA purines, it was essential that there should be negligible contamination of DNA with ribonucleotides. The precipitate of DNA from the alkaline digest of the mixed nucleic acids was dissolved in 10% aqueous sodium chloride solution and reprecipitated with alcohol, and the process repeated until the precipitate contained no ultraviolet absorbing material with a measurable R_F value on paper using 75% *iso*propanol as solvent in an atmosphere of ammonia. (Ribonucleotides and free purine and pyrimidine bases move under these conditions[13] while DNA remains on the starting line).

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In some experiments (see Tables) ribonucleotides were separated on columns of Dowex 2 (Cl^-)[14] and DNA bases, obtained by acid hydrolysis[15] were separated on Dowex 2 using alkaline chloride solvents[16], while in others, the respective purine bases were separated on paper chromatograms using 75% *iso*propanol/2 *N* HCl[17]. The acid-soluble fraction was investigated by the methods previously described[10].

RESULTS

One fact that emerges clearly from Tables I to III is that the change from a resting liver (with a high specific activity associated with both the acid-soluble nucleotides and with the ribonucleic acid purines) to a regenerated liver allowed the newly formed desoxyribonucleic acid purines to incorporate radioactivity from their environment.

The resting liver was removed 24 hours following the last injection of adenine, and BENNETT[18] has shown that less than 0.03% of adenine injected into the C_{57} mouse remains as free adenine after this period.

TABLE I

SPECIFIC ACTIVITY OF ACID-SOLUBLE ADENINE, AND OF RIBO- AND DESOXYRIBONUCLEIC ACID PURINES OF RESTING AND REGENERATED LIVER FOLLOWING INJECTIONS (0.2 mmole/kg/day for 3 days) OF ADENINE-8-^{14}C ($9.55 \cdot 10^4$ c.p.m./μmole) 3 rats 741 g. (All separations by paper chromatography).

	Acid-soluble adenine	*Ribonucleic acid*		*Desoxyribonucleic acid*	
		Adenine	*Guanine*	*Adenine*	*Guanine*
Resting liver (24 hours after last injection)	14100	8270	1880	240	50
Regenerated liver (48 hours later)	2700	4930	1710	1460	250

TABLE II

SPECIFIC ACTIVITY OF RIBO- AND DESOXYRIBONUCLEIC ACID CONSTITUENTS, AND OF THE VARIOUS ACID SOLUBLE NUCLEOTIDE FRACTIONS OF RESTING AND REGENERATED RAT LIVER FOLLOWING INJECTIONS (0.2 mmole/kg/day) for 3 days of adenine-8-^{14}C hydrochloride ($1.075 \cdot 10^5$ c.p.m./μmole): 6 rats, 801 g. (All separations, except the acid-soluble fractions, by paper chromatography.)

	Acid soluble fraction			*Ribonucleic acid*		*Desoxyribonucleic acid*	
	Monophosphate fraction	*Diphosphate fraction*	*Triphosphate fraction*	*Adenine*	*Guanine*	*Adenine*	*Guanine*
Adenine* (if present)	(1490)						
Resting liver (24 hours after last injection)	9000	6300	—	6680	1820	1100	50
Adenine* (if present)	(2050)						
Regenerated liver (48 hours later)	2550	1730	—	3490	1440	2020	650

* See discussion.

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TABLE III

SPECIFIC ACTIVITY OF RIBO- AND DESOXYRIBONUCLEIC ACID CONSTITUENTS OF RESTING AND REGENERATED RAT LIVER FOLLOWING INJECTIONS (0.2 mmole/kg/day for 3 days) OF ADENINE-8-^{14}C HYDROCHLORIDE (Two exp. 7.79 and $9.30 \cdot 10^4$ c.p.m./μmole): 3 rats — 810 g and 5 rats — 1198 g respectively. (All separations by ion exchange chromatography.)

	Ribonucleic acid				*Desoxyribonucleic acid*			
	Adenylic acid		*Guanylic acid*		*Adenine*		*Guanine*	
	Exp. A	*Exp. B*	*Exp. A*	*Exp. B*	*Exp. A*	*Exp. B*	*Exp. A*	*Exp. B*
Resting liver (24 hours after last injection)	5820	4860	1300	1300	515	270	760	130
Regenerated liver (48 hours later)	3580	3170	750	890	1350	1700	980	300

The acid-soluble fraction was not investigated in the above preliminary experiments.

Since the tissue regeneration does not involve mitosis for a further 24 hours[19] at least 40 hours will probably have elapsed between the last injection of free adenine and the onset of desoxyribonucleic acid synthesis. The change of the originally injected adenine being available for desoxyribonucleic acid synthesis after this interval cannot therefore be rated high.

In the experiment outlined in Table II, an attempt was made to identify free adenine in the ion exchange separation of the acid-soluble fraction. 0.01M NH_4Cl eluted a small amount of an ill-defined material with the spectral properties of adenine although it did not behave like adenine when subjected to ionophoresis on filter paper at pH 3.5. However, if the material were adenine, its specific activity was of the low order shown. The only radioactive materials likely to be present in any quantity are the general polyribonucleotides of the internal organs, to a lesser extent the polydesoxyribonucleotides of tissues undergoing constant cell division, urinary allantoin, and the acid-soluble nucleotides. At the low dosage given, 2:8-dihydroxyadenine, which accumulates in kidneys only during high doses of adenine[20], is unlikely to be present to any great extent. The suggestion is made that the radioactivity associated with the newly formed desoxyribonucleic acid had its origin in the acid-soluble nucleotides present. That it is less likely to arise from ribonucleic acid purines is suggested by the work of COHEN[21] studying labelled phosphate incorporation by bacteriophages; of SCHMIDT, HECHT AND THANNHAUSER[22] who were unable to confirm previous reports of BRACHET[23] that the increase in desoxyribonucleic acid was paralleled by a simultaneous decrease in the ribonucleic acid during sea urchin development; of VILLEE, LOWENS, GORDON, LEONARD AND RICH[24] studying the uptake of labelled phosphate by sea urchin eggs during development; and of ABRAMS[25] on the uptake of acetate and glycine by the same organism, (the dangers of arguing from one species to another being borne in mind). Of possible significance in this connection are the observations of MITCHELL[26, 27, 28] who showed that therapeutic doses of X- and γ-radiation on tumour tissue inhibited the synthesis of desoxyribonucleic acid. At the same time, with doses up to 1000 r, the ultraviolet absorption of the cytoplasm often markedly increased due, possibly, to the accumulation of pentose nucleotides. It would accord with the results obtained here if these nucleotides were, in fact, desoxyribonucleic acid precursors accumulating due to the inhibition of desoxyribonucleic acid synthesis.

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However, if acid-soluble nucleotides (mostly adenosine-5′-monophosphate in rat liver) are, indeed, utilised for desoxyribonucleic acid synthesis, it is surprising that *L. casei* is almost completely unable to utilise adenosine-5′-monophosphate for nucleic acid synthesis[29].

In the experiment detailed in Table II, the uptake of adenine into the desoxyribonucleic acid of resting liver was considerably higher than expected. A possible explanation is that the rats available at this time were much younger than those used in the other experiments.

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SUMMARY

Partial hepatectomy was performed on normal adult male rats which had received injections of adenine-8-^{14}C. Analysis of the liver removed showed that, as expected, the incorporated ^{14}C was associated with the RNA purines and with the acid-soluble nucleotides almost to the exclusion of the DNA purines. The cell division associated with the liver regeneration which followed, allowed the newly formed DNA to incorporate radioactivity into its purines from its environment. It is suggested that the acid-soluble nucleotides acted as DNA purine precursors under these conditions.

RÉSUMÉ

Des rats normaux, adultes, mâles, ayant reçu des injections d'adénine-8-^{14}C, furent soumis à des hépatectomies partielles. L'analyse des foies enlevées montrait, comme on pouvait s'y attendre, que le ^{14}C incorporé était associé avec les purines du RNA, et les nucléotides, solubles dans les acides et cela presque à l'exclusion des purines du DNA. Lors de la régéneration qui suivit le partage cellulaire permettait au DNA, nouvellement formé, d'incorporer, dans ses purines de la matière radioactivé du milieu environnant. Ces résultats conduisent à la conception que les nucléotides, solubles dans les acides, agissent, sous les conditions indiquées, comme précurseurs des purines du DNA.

ZUSAMMENFASSUNG

Partielle Hepatectomie wurde an normalen, ausgewachsenen, männlichen Ratten, die Injektionen von Adenin-8-^{14}C erhalten hatten, ausgeführt. Die Analyse der entfernten Leber zeigte, wie erwartet, dass das eingebaute ^{14}C mit den RNA-Purinen und den säurelöslichen Nukleotiden beinahe unter Ausschluss der DNA-Purinen verknüpft war. Die mit der nach folgenden Leberregeneration verbundene Zellteilung gab der neu gebildeten DNA die Möglichkeit radioaktive Bestandteile aus der Umgebung in die Purine einzubauen. Es wird vermutet dass unter diesen Bedingungen die säurelöslichen Nukleotide als Vorgänger der DNA-Purine auftreten.

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