contre, il est dépourvu de Cys, Met, Arg, His et d'acides aminés aromatiques. Les compositions en acides aminés du "NPN-12 %" de caséine entière, du "NPN-12 %" de caséine α, de B_α et de C_α sont indiquées dans le Tableau I.

Il apparaît donc que l'action de la présure sur la caséine entière comme sur la caséine α libère une substance dont la partie peptidique est la même. Cette partie peptidique forme environ 60 %, du "NPN-12 %" de caséine entière et du "NPN-12 %" de caséine α; elle ne forme qu'une proportion plus réduite des fractions B_α et C_α. L'étude des composés de la partie non peptidique (sucres, osamines, acide neuraminique, etc. . . .) est en cours. C'est sans doute cette dernière — et non la partie peptidique dont la composition en acides aminés semble constante — qui détermine le comportement chromatographique des diverses fractions.

Après action du réactif de SANGER sur le glycopeptide, aucun acide aminé N-terminal n'a pu être déterminé jusqu'à présent.

Par action de la carboxypeptidase (pH 7.5; 37°, présence de diisopropylfluorophosphate) plusieurs acides aminés sont rapidement détachés; mais après une action enzymique de 4 min seulement, on n'obtient que Val, Ala, et Thr avec un fort rendement. L'étude des peptides de l'hydrolysat trypsique (qui est actuellement en cours) permettra de déterminer exactement l'enchaînement C-terminal.

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Reçu le 4 may, 1959

Distribution of methylated purines in cell fractions from mouse liver and tumour

The occurrence of methylated purines as minor components of RNA has recently been reported by a number of workers[1,4,5,8,12]. In most of these investigations, analyses have been made on RNA from whole tissues or organs. In the work reported here we have found that the "soluble" cytoplasmic fraction of mouse liver and of a mouse mammary adenocarcinoma contains substantially more of these bases than mitochondrial or microsomal fractions, and also that the soluble cytoplasm RNA from tumour is richer in these bases than that from liver.

Tumour tissues and livers from tumour-bearing mice were homogenised in 0.25 *M* sucrose — 0.05 *M* Tris, pH 7.8. After centrifugation at 2,000 × *g* for 5 min, the mitochondrial fraction was sedimented by centrifugation at 10,000 × *g* (R_{max}) for 15 min in the Spinco model E ultracentrifuge. The microsome fraction was sedimented by

Abbreviations: RNA, ribonucleic acid; Tris, tris(hydroxymethyl)aminomethane; 1MeG, 1-methylguanine; $(Me)_1G$, 2-methylamino-6-hydroxypurine; $(Me)_2G$, 2-dimethylamino-6-hydroxypurine; 2MeA, 2-methyladenine; $(Me)_1A$, 6-methylaminopurine; $(Me)_2A$, 6-dimethylaminopurine.

a further centrifugation at 144,700 × *g* for 60 min. The soluble ribonucleoprotein and the mitochondrial and microsomal pellets were resuspended in the homogenisation medium, then precipitated by the addition of 2 vol. ethanol at pH 4, with the addition of $MgSO_4$ to 0.001 *M*. The precipitates were extracted by a modification of the procedure of MATTHEWS[11]. We shall present evidence in a subsequent report to show that this procedure removes all nucleotides except those that are components of polynucleotide sequences. The extracted pellets were hydrolysed with 2 *N* KOH for 22 h at 20°, neutralised with $HClO_4$, and chromatographed as bands in isopropanol-ammonia[10]. The nucleotides plus an area of paper down to, but excluding, the position of guanosine were eluted and purine nucleotides hydrolysed with 1 *N* HCl for 1 h at 100°. Measured quantities of hydrolysate were run in isopropanol-HCl[13] followed by butanol-NH_3[5]. In this system, 1MeG runs slightly ahead of guanine in the second dimension, while the Me_1G and Me_2G run adjacent to adenine. We located the 1MeG and combined Me_1G–Me_2G spots by their acid fluorescence in u.v. light. The 1MeG eluates from a number of chromatograms were combined, concentrated to a small volume, and run in butanol-NH_3 in the first dimension (12 h) followed by butanol-formic acid[5] in the second (30 h). The Me_1G–Me_2G areas were eluted and chromatographed two-dimensionally using isopropanol-HCl in both first and second dimensions. To isolate the methylated adenines we cut out an area of paper from each chromatogram that would include such compounds, combined the eluates, and re-ran in isobutanol-NH_3. The methylated purines were identified by comparison of their R_F's in various solvents and their acid and alkaline spectra with those of authentic synthetic samples of the bases. The distribution and approximate amounts of the methylated purines in our digests are presented in Table I.

We have not yet been able to isolate the isomeric nucleoside 2'- and 3'-phosphates from alkaline hydrolysates for all the bases listed in Table I. However, by a combination of paper electrophoresis in formate buffer at pH 2.0 and chromatography in isopropanol–NH_3 and satd. $(NH_4)_2SO_4$–sodium acetate–isopropanol[10] we have isolated pairs of substances with the expected properties of the 2-methylguanosine-2'- and 3'-phosphates and the 2-dimethylguanosine-2'- and 3'-phosphates.

As far as the distribution of these bases among the subcellular fractions is con-

TABLE I

APPROXIMATE AMOUNTS OF METHYLATED PURINE BASES IN LIVER AND TUMOUR SUBCELLULAR FRACTIONS

All data in moles/100 moles uracil. For the calculation of the amount of 1MeG, the extinction coefficient of guanine was used. The molar extinction coefficients for the other bases were: Me_1G, 1.2×10^4 at 252 mμ[7]; Me_2G, 1.4×10^4 at 252 mμ[7]; 2MeA, 1.06×10^4 at 263 mμ[8]; Me_1A, 15.1×10^3 at 267 mμ[8]; and Me_2A, 15.0×10^3 at 272 mμ[8].

	1MeG	1MeA	2MeA	Me_1G	Me_2G	Me_2A
Tumour mitochondria	— *	—	—	0.24	0.75	< 0.15
Liver mitochondria	—	—	—	< 0.10	0.57	< 0.10
Tumour microsomes	< 0.10	< 0.10	< 0.10	0.14	0.82	< 0.10
Liver microsomes	< 0.10	< 0.10	0.11	0.09	0.60	< 0.10
Tumour soluble	< 0.10	1.20	1.11	1.05	12.40	3.20
Liver soluble	< 0.10	0.75	0.55	0.80	5.20	1.30

* Not tested.

cerned, our results with mouse liver are in general agreement with those of SMITH AND DUNN[12] for rat liver. The soluble fraction is much richer in the methylated bases than the microsomes or mitochondria. We have found even higher amounts of these bases in the soluble fraction from the tumour. A base such as $(Me)_1G$ present as about 3% of the total RNA in the tumour soluble fraction is unlikely to be a contamination, or to be the result of a "synthetic accident" as was suggested as one possibility by DUNN AND SMITH[5] for bases occurring in very low amount. However, at least some of these methylated purines found in mitochondrial and microsomal RNA fractions may be present because these fractions are contaminated with small amounts of soluble RNA, especially as we have not yet examined carefully washed mitochondria and microsomes.

Liver was chosen as an easily accessible organ for comparison with tumour, but a more valid control would be a normal tissue with a cell-division rate comparable to that of the tumour. Furthermore, we have preliminary evidence suggesting that the difference between tumour and liver with respect to the methylated bases is greatest in young tumour. As the tumour ages, the proportion of methylated bases appears to decrease, and substantial changes in the ratios of the four major bases also occur. Thus, although the differences between tumour and liver soluble fraction shown in Table I are substantial, their biological significance remains to be determined.

We wish to thank Dr. J. F. BURTON for providing animal material. The tumour was a spontaneous mammary adenocarcinoma which has been maintained in his laboratory since July 1957. We also thank Drs. J. D. SMITH and D. B. DUNN for sending us a manuscript of their paper[12] before publication and for donations of methylated purines. This work was supported in part by a grant from the Rockefeller Foundation.

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Received May 27th, 1959