PARTICULATE COMPONENTS OF NORMAL AND TUMOR CELLS

PREVIOUS work in this laboratory has shown that a material, composed of small granules of uniform chemical constitution, could be isolated from both normal and tumor extracts by differential centrifugation at high speed.[1] The procedure adopted for the concentration and purification of these tissue fractions consisted essentially in a series of three to four alternate long and short runs in a high-speed centrifuge under a field of about 18,000 times gravity.[2]

If it is assumed that particles of all sizes are originally represented in the tissue extracts, it can be calculated that the above method will bring about the concentration of those particles ranging in size between 50 and 200 mμ in diameter (approximate density = 1.3). Of these, the larger particles are discarded at a much greater rate than the smaller ones during the short centrifugal runs so that the purified tissue fractions may be composed of a population of granules of various sizes, the largest being approximately three to four times the size of the smallest, with a predominance of particles with a diameter of 50 to 150 mμ. In dark-field illumination, the material prepared from various sorts of tissues appears to be composed of granules of nearly the same size. On the other hand, lack of homogeneity as regards particle size is apparent from the spreading of the boundary when the material prepared from both chicken tumor I and chick embryo is examined in the analytical centrifuge.[3]

The present note deals with a comparative study of material prepared, by the centrifugation technique, from chick embryo, mouse embryo, chicken tumor I, a spontaneous mouse tumor, and a transplantable sarcoma originally induced in a pure line of mice by the subcutaneous injection of benzpyrene. The material isolated from these various sources is strikingly similar and the purified fractions obtained from mouse embryo or mouse tumors have been found to possess many of the physical and chemical characteristics already described for the homologous fractions previously obtained from chick embryo and chicken tumor I.[4] Therefore, the properties of these different purified fractions can be discussed together.

No appreciable differences have been found in the solubility of the materials. The purified substance forms opalescent solutions at pH 7. In slightly acid solutions, a point of minimum solubility is found near pH 3.5, whereas the normal opalescence of the solution is appreciably decreased in solutions more alkaline than pH 11. The material is not precipitated from neutral solutions by heating or by 80 per cent. alcohol. The purified tissue fraction possesses a strong absorbing power for ultra-violet light, a maximum of absorption being found at or near λ2,600.

A marked similarity was found to exist, likewise, in the chemical constitution of the granules, irrespective of the tissues from which they were extracted. Chemical analysis shows them to be composed essentially of two main portions, one lipoid, the other protein in nature. A detailed study of these two major constituents of the purified tissue fraction has been made on previous occasions.[5] The lipoid fraction is found to be represented mainly by phospholipids, and to exhibit the properties of aldehydes, as shown by a strongly positive test with the fuchsin-sulfurous acid solution of Schiff.[6] The protein fraction appears to be represented chiefly by a nucleoprotein of the ribose type.[7]

In the present study, a pentose nucleic acid has been prepared from the protein portion derived from chick embyro, mouse embryo and mouse tumors, by following the procedure adopted previously with respect to chicken tumor material.[8] The nucleic acid obtained from any of these sources has been found to represent

[1] A. Claude, *Jour. Exp. Med.*, 66: 59, 1937; *Am. Jour. Cancer*, 30: 742, 1937; SCIENCE, 87: 467, 1938; *Proc. Soc. Exp. Biol. Med.*, 39: 398, 1938; SCIENCE, 90: 213, 1939.

[2] *Ibid.*

[3] K. G. Stern and F. Duran-Reynals, SCIENCE, 89: 609, 1939; A. Rothen and A. Claude, unpublished experiments.

[4] See footnote 1.

[5] *Ibid.*

[6] These lipoid components correspond probably to the group of acetalphosphatides recently described by R. Feulgen and Th. Bersin, *Z. Physiol. Chem.*, 260: 217, 1939.

[7] See footnote 1.

[8] A. Claude, SCIENCE, 90: 213, 1939.

TABLE I

CHEMICAL COMPOSITION OF THE PURIFIED MATERIAL (COMPLETE) ISOLATED BY DIFFERENTIAL CENTRIFUGATION

Source of material	N	P	C	H	Ash (total)	Ash (less P)	Lipoids (total)	Amount purified fraction in tissues (dryweight)
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Chick Embryo	8.22	2.10	59.54	8.65	6.36	1.53	51.0	12.4
Chicken Tumor I.....	8.60	1.54	59.96	8.99	6.00	2.46	36.5	2.9
Mouse Embryo	8.54	2.07	54.63	8.47	7.42	2.66	46.0	9.1
Mouse Sarcoma No. 180 (spontaneous)	8.00	1.52	56.34	8.90	5.32	1.83	49.1	6.6
Mouse Sarcoma No. 1549 (induced)	9.26	1.88	53.56	8.07	8.06	3.74	42.4	7.2

about 15 to 17 per cent. of the protein portion of the purified fraction.

Tables I and II give the results of chemical analysis of the purified fractions before and after extraction

TABLE II

CHEMICAL COMPOSITION OF THE PURIFIED MATERIAL (LIPOID-FREE) ISOLATED BY DIFFERENTIAL CENTRIFUGATION

Source of material	N per cent.	P per cent.	C per cent.	H per cent.	Ash (total) per cent.	Ash (less P) per cent.
Chick Embryo	13.80	1.21	49.92	7.02	4.07	1.27
Chicken Tumor I ..	12.74	1.16	48.52	7.29	5.94	3.24
Mouse Embryo ...	14.30	1.37	....	...	4.82	1.67
Mouse Sarcoma No. 180 (spontaneous)	14.51	1.21	49.32	6.84	4.23	1.45
Mouse Sarcoma No. 1549 (induced) ..	14.90	1.23	49.77	6.70	4.51	1.68

with organic solvents. In the present experiments, the purified material was found to represent 3 to 7 per cent. of the tumor tissues and as much as 9 to 12 per cent. of the combined tissues derived from the whole mouse and chick embryos.

The above observations indicate that particulate elements, present in normal and tumor tissues, have the general constitution of a phospholipid-ribonucleoprotein complex. The occurrence, in tissue extracts, of a complex of definite chemical composition raises the question whether or not these elements may preexist in the form of similar bodies in the protoplasm. That the structure of cellular components may persist through the process of purification is suggested by the fact that the preparation of the purified fraction is accomplished by purely mechanical means, and that the method was precisely devised with the view of preserving, as much as possible, the integrity of certain active elements of the cell.

The formed elements of the cell which, by their mass, represent an important part of the cellular body, are the nucleus, the Golgi apparatus and the chondriome. The fact that the purified materials appear to contain ribose nucleic acid only is taken to indicate that the particles are not fragments of the nucleus. Furthermore, the nuclei are usually discarded by the first centrifugation at low speed.[9] As regards the Golgi apparatus, it has been shown that the substance of this cellular component has a relatively low density and that it moves toward the centripetal pole when the tissue is submitted to high-speed centrifugation.[10] On the other hand, a review of the general properties of mitochondria indicates that these elements possess many important features in common with the constituents of the purified fractions.[11] According to Cowdry,[12] the breadth of mitochondria may vary, in different localities, from 50 to 200 mμ in diameter. Particles of this size are those which would be concentrated by our method of differential centrifugation at high speed.

The assumption that the granular elements of the purified tissue fraction may represent isolated mitochondria or fragments of mitochondria is also supported by the chemical nature of the material. It is now generally admitted that mitochondria are complex elements made in large part of phospholipids and proteins.[13,14] The occurrence together of nucleoproteins and of certain phospholipids, highly soluble in alcohol and presenting the properties of aldehydes, might explain the response of mitochondria to certain histological dyes and fixatives.[15] There is strong evidence that mitochondria play an important part in the differentiation of the cell.[16] The demonstration that the material isolated from normal and tumor tissues represents, in fact, part of the chondriome and the fact that at least one of these fractions possesses tumor-producing activity[17] should lead to interesting developments in the study of the chemical nature of mitochondria and their possible role in the evolution of the malignant cell.

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[9] R. R. Bensley and N. L. Hoerr, *Anat. Rec.*, 60: 251 and 449, 1934; also personal observations.

[10] H. W. Beams and R. L. King, *Anat. Rec.*, 59: 363, 1934.

[11] The literature on mitochondria is summarized in the monograph of Cowdry (footnote 12) and in that, more recent, of Guilliermond (footnote 14).

[12] E. V. Cowdry, Carnegie Institution of Washington, *Contrib. Embryol.*, 8: 39, 1918.

[13] *Ibid.*

[14] A. Guilliermond, "Les constituants morphologiques du cytoplasme," Hermann et Cie, Paris, 1934.

[15] Bensley and Hoerr (footnote 9) and Bensley (footnote 18) found that mitochondria preparations contained as much as 43 per cent. lipoids, but concluded that the predominating fats were glycerides, not phospholipins. They drew no conclusions regarding the nature of the proteins found in the preparations.

[16] F. Meves, *Arch. Mikr. Anat.*, 72: 816, 1908.

[17] See footnote 1.

[18] R. R. Bensley, *Anat. Rec.*, 69: 341, 1937.

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