Stimulation of DNA Synthesis with Histones from Tumor Tissues.* (27643)

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The presence of tumors lead to multiple changes in the metabolism of the host. Very often an extensive splenomegaly or increase in the size of the liver is noted. This increase in organ size is connected with an increase of mitotic activity in organs of tumor bearing animals and with the increase of DNA turnover(1). Injection of tumor homogenates or of crude tumor extracts into the animals leads also to an increase of mitotic activity and of DNA turnover in different organs(2,3). The chemical nature of a factor or factors in tumor tissues which are responsible for stimulation of cell division is little understood. Malmgren and Mills(4) suggested as a result of studies of the U.V. spectrum of such extracts, that the factor is a nucleic acid material. In an attempt to characterize the chemical nature of this material the following experiments were undertaken.

Material and methods. Histones and DNA were prepared from hepatoma 134 and C_3H/Sx mammary carcinoma maintained on C_3H/Crgl mice as host and from liver and spleen from healthy, as well as tumor bearing C_3H/Crgl mice. Other samples of histones were prepared from Rous sarcoma, from rat Walker carcinoma, from warts of rabbits with Shope papillomatosis and from approximately 12-day-old normal rat embryos.

The tissue was washed with 0.14 M sodium chloride containing 0.01 M sodium citrate by blending in a Servall omni-mixer and spinning at 900-1000 *g* for 20 minutes. After 4-6 washings the final sediment of crude deoxyribonucleoprotein was extracted with 0.2 N HCl for the histone preparation or dissolved in 1 M sodium chloride containing 0.01 M sodium citrate for the preparation of DNA. From 1 M sodium chloride solution DNA was prepared by deproteination according to Sevag *et al.*(5). The acid extract of the crude deoxyribonucleoprotein, containing histones, was clarified by centrifugation, dialyzed against distilled water and lyophilized.

Male C_3H/Crgl mice were injected intraperitoneally daily for 6 consecutive days with 0.3 ml of 0.1% histone solution in saline or with 0.3 ml of 1% DNA solution in saline. Six hours after the last infection the mice were injected intraperitoneally with 0.4 μC of H^3 thymidine in saline for every gram of body weight. Fourteen to 16 hours later the animals were killed and the amount of incorporation of H^3 thymidine in the livers, spleens and kidneys was determined. The DNA was extracted after a method published by Field *et al.*(6), and H^3 was determined in Packard Tri-carb liquid scintillation spectrometer. Specific activities of DNA were expressed as ratio between counts per minute and optical density at 260 mμ.

Results and discussion. The comparison between the rate of incorporation of H^3 thymidine in the DNA after the repeated injection of DNA from mammary carcinoma and after the repeated injection of histones from mammary carcinoma (Table I) shows that the factor responsible for the rise in incorporation is histone in nature. The increase in incorporation of H_3 thymidine into the spleen after injection of 10 times higher concentration of DNA was only one-half the increase which followed injection of histones. The Millon reaction(7) indicated that the DNA had about 5% of histones as a contamination and the increase in the incorporation after injection of DNA can be attributed to this contamination. Similar results as with DNA and histone from mammary carcinoma were obtained with DNA and histone from hepatoma.

It cannot be excluded that the activity of the liver mitotic stimulant(4) which had been characterized as nucleic acid material is caused by histones because the N:P ratio of 3.64 of this material indicates heavy protein contamination.

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TABLE I. Specific Activity of DNA after Intraperitoneal Injection of 0.003 μg Thymidine Methyl H_3 (Activity 0.4 μC) per Gram of Mouse Body Weight.

	No. of mice in experiment	Specific activity of DNA (cpm/O.D. 260 mμ) with stand. dev., in organs of animals inj. with material listed in left column		
		Spleen	Liver	Kidney
Saline	20	3242 ± 363	322 ± 39	351 ± 42
DNA from liver (healthy mice)	12	3228 ± 308	—	—
" " mammary carcinoma	12	4066 ± 214	290 ± 30	403 ± 39
Histone from mammary carcinoma	28	4780 ± 344	511 ± 63	526 ± 60
DNA from hepatoma	12	3513 ± 261	—	—
Histone from hepatoma	20	5013 ± 343	481 ± 38	489 ± 36
" " Rous sarcoma	12	4841 ± 581	463 ± 49	535 ± 69
" " Shope papillomatosis	16	4770 ± 271	491 ± 36	500 ± 47
" " Walker carcinoma	12	4751 ± 304	445 ± 48	455 ± 39
" " liver (healthy mice)	16	3540 ± 395	313 ± 47	394 ± 42
" " spleen (healthy mice)	8	3176 ± 252	326 ± 25	343 ± 32
" " normal rat embryos	12	3900 ± 322	398 ± 65	433 ± 35
" " Walker carcinoma*	8	3994 ± 267	427 ± 43	433 ± 25
" " liver†	14	3225 ± 365	322 ± 39	376 ± 37
" " spleen†	16	3012 ± 388	328 ± 42	415 ± 47

* After additional purification.

† Mice with mammary carcinoma.

DNA synthesis was stimulated not only with histones from mouse tumors but also, especially in the spleen, with histones from tumors of other species, *e.g.*, Rous sarcoma, Shope papillomatosis and Walker carcinoma (Table 1). This shows that the DNA synthesis stimulating factor is probably generally present in tumors and is not species-specific, It is also present in histones from embryonic tissues, but to much lesser extent. Histones from liver and spleen of healthy mice as well as from liver and spleen of tumor bearing mice were without any effect.

Because the histones prepared by acid extraction are still contaminated with traces of a protein with isoelectric point at pH 5.0, a group of mice was injected with histone from Walker carcinoma which has been additionally purified by extraction with acetone and repeated trichloracetic acid reprecipitation. Such purification led to some loss in DNA synthesis stimulation activity, but this might be due either to denaturation and consequent loss of biological activity of histones resulting from the chemical treatment, or to removal of some active fraction from the histone.

The question is still open whether histones from tumor tissues differ from those of normal tissues. Cruft *et al.*(8) found differences in chemical characteristics of both types of histones, yet other authors(9,10) have not been able to confirm this. Interesting are the experiments of Davis and Busch(11,12) who found in histones separated on carboxymethylcellulose a fraction with very high turnover rate, which was present only in histones isolated from tumor tissues. The existence of metabolically very active histone fraction present only in tumor tissues and the DNA synthesis stimulating activity of histones isolated from such tissues may not be a coincidence. Histones, because of their high positive electric charge, have the possibility of reacting with nucleic acids and thus possibly controlling the rate of their metabolic activity(13).

Summary. The histones isolated from different tumor tissues enhance the incorporation of H^3 thymidine into DA of various organs of C_3H mice, especially into spleen. When isolated from embryonic tissues, the histones promote such incorporation to a lesser extent. Additional purification of these histones leads to a decrease in this enhancement. The histones isolated from normal organs do not have any effect.

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Effect of Reserpine on Fatty Acid Mobilization.* (27644)

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The mobilization of free fatty acids (FFA) from adipose tissue is increased during fasting and in response to the exogenous administration of growth hormone, epinephrine and norepinephrine. In addition, corticotropin, thyrotropin and possibly growth hormone, as well as epinephrine and norepinephrine, are potent lipolytic agents when incubated with adipose tissue *in vitro* (see 1 for review). Because the mode of action of all of these agents is similar as judged by their effects on carbohydrate metabolism and oxygen consumption(1, 2), as well as their effects on FFA mobilization, the possibility arises that all of these agents may exert their action on adipose tissue through a common pathway or by stimulating the release of a common mediator within the adipose tissue. The recent finding that norepinephrine is present in significant amounts in adipose tissue(3,4) lends credence to the possibility that the lipolytic agents mentioned above might act simply by releasing the catecholamines bound presumably in nerve endings in adipose tissue. In a preliminary communication, Paoletti *et al.*(3) reported that not only could adipose tissue be depleted of norepinephrine by pretreating rats with reserpine, but that rats so treated failed to mobilize FFA in response to corticotropin.

The present experiments were performed in an attempt to assess the role of catecholamines in the FFA mobilization which results from fasting and growth hormone administration.

Materials and methods. Studies were performed using fed and fasted normal male rats of the Charles River strain weighing approximately 150 g. Blood was taken from the abdominal aorta for determination of plasma FFA concentration. Epididymal adipose tissue was removed and samples of the thin distal portions were weighed and placed in vials of Krebs-Ringer phosphate buffer containing 5% fat-free human serum albumin. The vials were incubated in a Dubnoff metabolic water bath at 37°C for 3 hours. Combined FFA content of the tissue plus incubation medium was determined in each case according to the procedure of Dole(5). Blood sugars were

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