Chemical Changes in Tumor-Bearing Mice.* (19743)

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It has previously been shown in this laboratory that significant changes occur in the nucleic acid content of the tissues of tumor-bearing mice(1-3). Similar changes, though less pronounced, were observed in mice during gestation(4). Evidence has also accumulated to show that the metabolic pattern of the organism as a whole is markedly altered by the presence of a malignant tumor(5,6). In view of the close relationship between the nucleic acids and protein synthesis during growth processes, it was considered of interest to study some of the amino acids in the tissues of sarcoma-bearing mice. A study of the individual purines and pyrimidines was also carried out, with a view of gaining some information regarding the composition of the tissue nucleic acids.

The data reported in the present paper show that along with the changes in the purine and pyrimidine bases, there occur alterations in the tissue proteins, as indicated by a significant increase in certain amino acids.

Experimental. Material and methods. Mice of the Swiss strain were employed in this study. Crocker sarcoma S-180† was transplanted subcutaneously into the right pectoral region of 5- to 6-week-old mice of mixed sexes. Groups of 40 animals were killed by decapitation at the end of 1, 2, and 3 weeks after implantation of the tumor. The separately pooled tissues were homogenized using distilled water at 0°C. (a) *Nucleic acids and purines.* The nucleic acids were extracted with hot trichloracetic acid(7). The pentose nucleic acid (RNA) was determined according to the method of von Euler and Hahn(8), and the desoxypentose nucleic acid (DNA) according to that of Stumpf(9). The remaining extracts were hydrolyzed with sulfuric acid (2), and guanine and adenine were determined according to the methods of Hitchings(10), and Woodhouse(11), respectively. (b) *Pyrimidines.* Estimation of pyrimidines was carried out as described previously(1), except that the chromatograms were developed in an iso-propanol-HCl medium(12). (c) *Amino acids.* An aliquot of each tissue homogenate, containing approximately 2 g of fresh tissue was adjusted with distilled water and concentrated HCl so that the final suspension had a volume of 25 ml and was 5 N in HCl. The tissues were hydrolyzed by autoclaving at 15 lb pressure for 8 hours. The hydrolysates were brought to a pH of 6.8 with NaOH solution and diluted to about 200 ml with a final concentration of approximately 2 mg of protein per ml. Arginine, histidine, and threonine were determined microbiologically with *Streptococcus faecalis*(13), while glycine was assayed with *Leuconostoc mesenteroides* P-60 (14). Cystine was determined colorimetrically by the method of Winterstein and Folin, as modified by Block and Bolling(15). The protein content was estimated by multiplying the Kjeldahl nitrogen value by 6.25.

Some difficulty was experienced in determining uracil in the lung, and thymine in the liver, since liver DNA and lung RNA are low. This difficulty was overcome by placing known amounts of standard solutions of uracil and thymine on the filter paper along with the hydrolysates. After developing the chromatograms the pyrimidine content of the tissues was determined by difference.

Results. In Tables I and II are presented the values for the nucleic acids, purines and pyrimidines in the sarcoma, and in the liver, lung, and kidney of sarcoma-bearing mice. The changes in the nucleic acids are reflected in the values for the purines and pyrimidines. Of significance are the changes in the pyrimidines, uracil, and thymine, which are a measure of RNA and DNA, respectively.

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† Obtained from the Jackson Memorial Laboratory, Bar Harbor, Me.

TABLE I. Changes in Nucleic Acids and Purines in a Sarcoma and in Tissues of Sarcoma-Bearing Mice.*

Tissue	Wk after implantation of tumor	mg/g dry wt RNA	DNA	Adenine	Guanine
Sarcoma	1	52.5	43.5		
	2	44.7	38.3		
	3	31.2	36.2		
Liver	Control†	33.5 ± .9	9.8 ± .8	3.1 ± .1	6.4 ± .3
	1	40.2	12.3	5.8	8.9
	2	38.4	11.8	4.8	8.1
	3	40.2	17	5	7.5
Lung	Control	8.6 ±1.5	17.5 ± 2.2	3 ± .7	3.3 ± .6
	1	16.9	26.5	2.4	4.7
	2	14.7	33.9	3.8	5.9
	3	21.9	37	3.9	7.2
Kidney	Control	24.6 ± .9	21.9 ± 1.9	3.3 ± .1	5.6 ± .3
	1	21.5	21.5	3.9	5.9
	2	22.1	25.6	4.3	7
	3	24.3	33.7	5.3	7.7

* Values for RNA and DNA are taken from a previous publication(1).

† Values for controls are means ± stand. errors. S.E. $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$, where d is the deviation from the mean and n the number of determinations.

TABLE II. Pyrimidine Content of a Sarcoma and of Tissues of Sarcoma-Bearing Mice.*

Tissue	Wk after implantation	Uracil, μg/100 mg dry wt	% of RNA	Cytosine, μg/100 mg dry wt	Thymine, μg/100 mg dry wt	% of DNA
Sarcoma	1	166	3.2	671	480	11
	2	138	3.1	577	425	11.1
	3	125	4	477	427	11.8
Liver	Control	113	3.38	284	92	9.4
	1	168	4.18	391	116	9.4
	2	160	4.17	386	107	9.1
	3	169	4.18	422	158	9.3
Lung	Control	44	5.1	109	163	9.3
	1	93	5.5	191	237	8.9
	2	80	5.4	212	298	8.8
	3	99	4.5	264	333	9.1
Kidney	Control	129	5.2	228	215	9.8
	1	120	5.6	240	208	9.7
	2	134	6.1	244	218	8.5
	3	112	4.6	260	256	7.6

* All values are averages of triplicate determinations on a single hydrolysate.

In general, the increase in uracil follows the increase in RNA values, and that of thymine corresponds to the changes in DNA. It will also be noted that in the kidney the RNA content remains unchanged. The fact that the uracil concentration in this organ shows no significant change over the control is further evidence that only the DNA concentration increases in this tissue. When the values for uracil and thymine are expressed (Table II) as per cent of RNA and DNA, respectively, there is fairly good agreement among the thymine values for the liver, kidney, and lung, amounting to approximately 9% of the estimated DNA content. The corresponding values for the sarcoma are higher (about 11% of DNA). The values for uracil, however, show large variations.

Table III shows the percentages of the amino acids in the sarcoma and in the liver, lung, kidney, and spleen of sarcoma-bearing mice. In analyzing these data any change

TABLE III. Percentage of Amino Acids in Sarcoma S-180 and in Tissues of the Host.*

Tissue	Wk after implantation	Methionine	Arginine	Histidine	Threonine	Glycine	Cystine
Sarcoma S-180†	1	1.75 ± .05	4.56 ± .07	1.84 ± .02	3.40 ± .10	3.96 ± .06	1.11 ± .08
	2	2.15 ± .05	6.55 ± .09	2.06 ± .01	4.24 ± .09	4.76 ± .08	1.24 ± .11
	3	1.80 ± .07	6.11 ± .13	1.88 ± .03	3.99 ± .14	4.91 ± .10	1.19 ± .05
Liver	0	1.87 ± .05	4.15 ± .03	1.62 ± .01	3.47 ± .03	3.81 ± .05	.82 ± .05
	1	2.20 ± .03	5.30 ± .12	2.14 ± .01	3.74 ± .10	5.02 ± .04	1.18 ± .04
	2	2.01 ± .04	5.66 ± .04	2.02 ± .02	3.98 ± .15	5.66 ± .05	1.02 ± .07
	3	2.03 ± .04	6.06 ± .16	2.21 ± .04	4.43 ± .10	5.73 ± .01	1.23 ± .09
Lung	0	1.40 ± .03	3.43 ± .05	4.16 ± .06	3.95 ± .05	4.07 ± .06	1.06 ± .08
	1	1.50 ± .01	4.94 ± .17	3.90 ± .03	3.94 ± .07	4.90 ± .03	1.29 ± .08
	2	1.42 ± .02	5.41 ± .05	3.88 ± .03	4.30 ± .05	5.92 ± .10	1.35 ± .02
	3	1.57 ± .03	5.90 ± .09	3.46 ± .05	3.93 ± .04	5.32 ± .05	1.24 ± .01
Kidney	0	2.03 ± .05	4.68 ± .03	1.99 ± .03	4.70 ± .06	5.28 ± .14	1.26 ± .06
	1	2.12 ± .01	6.15 ± .17	1.92 ± .04	4.42 ± .06	4.90 ± .03	1.22 ± .04
	2	2.11 ± .04	5.60 ± .09	1.92 ± .03	4.50 ± .11	5.65 ± .10	1.27 ± .01
	3	2.11 ± .05	6.38 ± .13	2.08 ± .02	4.78 ± .08	5.31 ± .12	1.31 ± .02
Spleen	0	1.43 ± .02	4.46 ± .05	2.53 ± .06	3.52 ± .08	5.30 ± .05	
	1	1.39 ± .03	4.87 ± .04	2.28 ± .04	3.62 ± .04	—	
	2	1.57 ± .08	4.67 ± .07	2.30 ± .06	3.50 ± .11	5.31 ± .10	
	3	1.55 ± .02	4.69 ± .06	2.50 ± .05	3.97 ± .10	5.55 ± .14	

* Amino acid values expressed as % of total protein, the amount of protein having been calculated on the basis of 16% nitrogen.

† Values for each group are means ± stand. errors. S.E. $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$, where d is the deviation from the mean and n is the number of determinations. "n" = 8 for all amino acids except cystine, where "n" = 6.

less than 15% is not considered significant. In the sarcoma, the significant change in arginine and glycine may be noted. The values for the second and third week for these amino acids are higher than those for the first week. More interesting, and perhaps of greater significance, are the changes in the amino acids in other tissues. In the liver, the changes in methionine are not considered significant, but all the other amino acids show a definite increase over the normal controls. The maximum increases are: arginine, 46%; histidine, 36%; threonine, 28%; glycine, 50%; cystine, 50%. In the lung, an increase of 72% in arginine, 46% in threonine, and 27% in cystine was found. On the other hand, there is a progressive drop in the histidine content of the lung as the tumor develops. Arginine alone shows a significant increase in the kidney, whereas in the spleen none of the amino acids studied show a significant change.

Discussion. The changes observed in the pyrimidines and purines confirm our previous findings(1-3) that there is a significant increase in the nucleic acid content of the tissues of tumor-bearing mice. The increases in uracil and thymine parallel the observed changes in RNA and DNA, respectively.

It was hoped that the determination of the individual components of the nucleic acids would give some indication of the composition of the 2 nucleic acids in the various tissues. However, the values for uracil when expressed as per cent of RNA vary considerably in the different tissues. It must be emphasized that unless the 2 nucleic acids are separated, and the composition of each studied separately no rigid conclusions can be drawn. We plan to extend our studies along these lines. It is noteworthy that there is generally (except for the third week value for kidney, Table II) good agreement in the thymine values of the liver, lung, and kidney, when expressed as per cent of DNA. The values for the sarcoma are consistently higher. It will be noted that the nucleic acids were estimated colorimetrically by methods which are not highly sensitive. Secondly, the results are expressed on the basis of the dry weight of the tissue. Both of these methods leave much to be desired. In view of these limitations the agreement in the thymine values is remarkable. It is not

yet clear whether the composition of the nucleic acids in the sarcoma is different from that of other tissues. But in the case of the liver, lung, and kidney, it is concluded that the composition of DNA is the same. In this context the work of Chargaff and coworkers (16) should be mentioned. Their observations on isolated samples of DNA from a variety of sources suggest that the composition of this nucleic acid is constant for different organs of the same species and characteristic of the species.

The increases in amino acids might be ascribed to an increase either entirely in the free amino acids, in the protein alone, or in both. Preliminary studies on the liver in this laboratory‡ thus far indicate that the increase in the amino acids is not solely due to an increase in the free amino acids. Significant increases in the amino acids (including free amino acids) have been found to occur in neoplasms and in the tissues of newborn mice (17,18).

The fact that the amino acids in the spleen show no significant change may have a bearing on our previous observations(1-4) that the nucleic acid content of this organ does not show a definite increase as in the case of the other tissues.

Summary. 1. The nucleic acids, purines, pyrimidines, and methionine, arginine, histidine, threonine, glycine, and cystine have been determined in various tissues of mice of the Swiss strain bearing transplanted sarcoma 180 (Crocker). 2. The changes in the nucleic acid content of the tissues are paralleled by the values for the individual purines and pyrimidines. In the liver and lung of mice bearing sarcoma, there is an increase in both RNA and DNA, and this increase is further confirmed by the corresponding increases in uracil and thymine. The fact that only the DNA content of the kidney increases is further supported by an increase in the thymine values alone, while the uracil content of this organ remains unchanged. 3. The values for thymine when expressed as per cent of DNA, suggest that the composition of DNA in the liver, lung, and kidney is the same. 4. The study of the amino acids present in a sarcoma and in the tissues of normal and sarcoma-bearing mice reveals that there are significant changes in the amino acids of the tissues of tumor bearing animals. In the sarcoma, there is a significant increase in arginine and glycine, the values for the second and third week being higher than those for the first week. In the livers of these animals, there is a significant increase in arginine, histidine, threonine, glycine, and cystine over the normal controls. In the lung, the increase is noted in arginine, threonine, and cystine, while there is a drop in the histidine content, as the tumor progresses. Arginine alone shows a significant increase in the kidney, while in the spleen none of the amino acids studied shows any change over the normal control values.

‡ Experiments by Mr. E. J. Singer.

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