ity of the isolated ileum was not affected by cortisone acetate treatment *in vivo* or *in vitro*.

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Nucleic Acid Changes in Tumor-bearing Mice.* (19181)

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Reddy and Cerecedo(1) observed a marked rise in the nucleic acid content of the tissues of mice bearing transplanted Sarcoma 180, and of those with transplanted melanoma S-91. An increase in the liver desoxypentose nucleic acid (DNA) fraction of rats fed p-dimethylaminoazobenzene, long before any hepatoma developed, was found by Masayama and Yokoyama(2). These findings have been recently essentially confirmed by Price *et al.* (3). In this laboratory, similar changes were observed in the tissues of mice during gestation(4).‡

In this paper we report data showing a relationship between the rate of growth of the tumor and the changes in the nucleic acid content of certain organs in the host. We also present data on the nucleic acid, purine, and pyrimidine content of melanoma S-91 at different stages of growth.

Materials and methods. Two strains of mice, dba and the Swiss, were employed in this study. The tumors, Sarcoma 180 and malignant melanoma S-91 were obtained from The Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. They were transplanted subcutaneously into the right pectoral region of 5- to 6-week-old mice of mixed sexes. Groups of 4, 5 or more animals were killed by decapitation at given intervals. The pooled tissues were homogenized at 0°C, and the nucleic acids extracted with hot trichloroacetic acid(5). The pentose nucleic acid (RNA) was determined according to the method of von Euler and Hahn(6), and the DNA according to that of Stumpf(7). Guanine and adenine were determined according to the methods of Hitchings(8), and of Woodhouse(9), respectively, as used in the combined procedure of Lombardo and Cerecedo (10). Uracil and cytosine were prepared in this laboratory. Thymine was generously supplied by The Fleischmann Laboratories, New York, through the courtesy of Dr. F. J. DiCarlo. The pyrimidines were estimated by means of paper chromatography. Since the

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‡ After preparing the manuscript of this paper, our attention was called to a publication of Kelly and Jones(19), who studied the turnover of desoxypentose nucleic acid in mice bearing transplants of Strong's mammary carcinoma. Their results are in harmony with our findings.

TABLE I. Nucleic Acid Changes in the Tissues of dba Mice Bearing Sarcoma S180.

Tissue	Controls (22;5)*	1st wk (22;5; 1.09)*	2nd wk (30;3; 7.94)*	3rd wk (12;2; 16.2)*
		RNA, mg/g dry wt		
Sarcoma		48.5 ± 2 †	44.3 ± 1.03†	44.3 ± 1.90†
Liver	30.2 ± .20	30 ± .46	41.5 ± 2.19	52 ± 1.79
Kidney	26.2 ± .70	22.8 ± 1.77	27.4 ± 1.41	26.2 ± .25
Lung	10.2 ± .56	11.8 ± .71	20 ± .32	16.9 ± .30
Spleen	46.4 ± .59	42.6 ± 1.56	47.1 ± .24	51.4 ± 1.05
		DNA, mg/g dry wt		
Sarcoma		35.8 ± 7.06	48.6 ± 3.96	37.5 ± 1.53
Liver	9.6 ± .44	10.3 ± .83	15.6 ± 1.99	20.6 ± .30
Kidney	21.7 ± .78	27.7 ± 1.28	32.5 ± 1.43	31.4 ± 1.10
Lung	29.8 ± .72	24.5 ± 1.90	36.2 ± 1.78	46.2 ± 6.20
Spleen	163 ± 11.16	177.2 ± 3.25	117.8 ± 9.67	155.5 ± 1.50

* The first No. in parenthesis indicates No. of animals used; the second the No. of determinations; the third the wt of tumor expressed as % of body wt.

† All values are means ± standard errors S.E. $= \sqrt{\frac{\Sigma(x)^2}{n(n-1)}}$.

TABLE II. Nucleic Acid Changes in Tissues of Swiss Mice with Transplantable Sarcoma.

Tissue	Controls (72;5)*	1st wk (49;1; .6)*	2nd wk (40;1; 3.40)*	3rd wk (40;1)*
		RNA, mg/g dry wt		
Sarcoma		52.5	44.7	31.2
Liver	33.5 ± .9†	40.2	38.4	40.2
Kidney	24.6 ± .9	21.5	22.1	24.3
Lung	8.6 ± 1.5	16.9	14.7	21.9
Spleen	40.7 ± 1.6	39.6	36	36.2
		DNA, mg/g dry wt		
Sarcoma		43.5	38.3	36.2
Liver	9.8 ± .8†	12.3	11.8	17
Kidney	21.9 ± 1.9	21.5	25.6	33.7
Lung	17.5 ± 2.2	26.5	33.9	37
Spleen	141.1 ± 18.5	103	89	90.5

* The first No. in parenthesis indicates No. of animals used; the second the No. of determinations; the third the wt of the tumor as % of body wt.

† All values are means ± standard errors.

procedure used in our experiments is a modification of the methods of Hotchkiss(11), and of Vischer and Chargaff(12), it will be described in some detail. After removal of phospholipids and of acid-soluble phosphorus the tissue (60-100 mg dry wt) was treated with 70% perchloric acid(13). Chromatograms were developed on strips of Whatman No. 1 filter paper, using two different solvent systems. In one case, strips were developed with a mixture of 14% water in n-butanol (v v)(11). This system gave a clean separation of guanine (Rf 0.12), cytosine (Rf 0.22), and thymine (Rf 0.48), but no clear separation of uracil and adenine. The second system consisted of a mixture of 7 volumes of n-butanol and 1 volume of 1 N HCl. This effected a separation of uracil (Rf 0.32) and thymine (Rf 0.45). The position of the substances was determined by observation of the chromatogram under ultraviolet light(13). Cytosine and uracil were eluted with 5 ml of distilled water and determined colorimetrically(14). Thymine was eluted with 0.1 N HCl and then estimated spectrophotometrically(12).

Results. The results of the experiment with sarcoma 180 in the 2 strains of mice are summarized in Tables I and II. They show that in the dba animals, (Table I) there are no changes in the nucleic acid content of any of the organs studied at the end of the first week. Two weeks after transplantation, there is a pronounced rise in the RNA and DNA of the liver and the lung, whereas in the kidney, only the DNA shows this increase. On the other

TABLE III. Nucleic Acid Changes in Tissues of Mice (dba) Bearing Melanoma S-91.

Group	No. wk after inoc.	No. of animals	Tumor wt as % of body wt	Liver RNA mg/g dry wt	Liver DNA mg/g dry wt	Kidney RNA mg/g dry wt	Kidney DNA mg/g dry wt	Lung RNA mg/g dry wt	Lung DNA mg/g dry wt	Melanoma RNA mg/g dry wt	Melanoma DNA mg/g dry wt
I	4	5	.65	29.1	12.6	27.2	24.4	11.7	28.7	56.8	55.1
	5	5	.79	29.8	11.3	24.8	24.6	11.4	27.4	49.2	57.2
	6	5	4.78	38.6	14.9	27	35	11.1	29.4	50.5	64.1
	7	4	10.15	34.2	17.1	23.6	38.4	16.4	30.8	43	66
	8	4	13.10	37.4	17.4	23.8	32.8	17	37.4	42.7	61.5
	9	4	11	43.6	17.7	22.4	27.4	18.3	54	23.2	44
II	4	5	.58	30.2	10.2	26.2	18.8	10.5	27.3	56	48
	5	5	.67	32.6	11	24.8	20	8	21.2	57.2	44.5
	6	5	1.80	30.6	9.6	25.8	20	11.1	25.8	54.3	44.2
	7	5	2.26	35.7	8.4	27.7	22.5	13.1	29	49.7	34.4
	10	4	9.26	37.6	12	24.2	36.2	12.8	38.5	50.2	54.9
	12	4	9.95	35.6	12	22.4	33.8	15	32.8	43	54.3

hand, in the Swiss strain (Table II) the changes in the liver and lung are significant after the first week. If the maximum increases are compared, we find that in the dba mice, the content of RNA in the liver has increased by 72%, and of DNA by 115% over the controls. The corresponding increases in the liver of the Swiss mice are 20% and 73%. On the other hand, the increases in the lung nucleic acids in the Swiss mice are considerably higher than in the dba mice. Apparently, we are dealing here with a strain difference.

The experiments with the melanoma were performed on 3 different occasions. In the initial experiment, approximately 10-week-old animals were used. The tumor was palpable after 3 weeks, but thereafter its growth was slight and irregular. As there were no changes in the nucleic acids at the end of 8 weeks, the experiment was discontinued. The experiment was repeated twice. A 6-week-old tumor was transplanted into 5- to 6-week-old animals. In spite of the fact that the conditions under which the experiments were carried out were the same, the growth of the tumor and the changes in the tissue nucleic acids were different in the 2 experiments. Therefore, the data are given separately in Table III. In one experiment (Table III, Grp. I) significant changes were first observed in the liver at the end of 6 weeks, when the tumor size was 4.8% of the body weight. Thereafter, a progressive increase in the RNA and DNA of the liver and lung was observed. The kidney DNA behaved somewhat differently. There was a sudden increase of 61% at the end of 6 weeks; of 77% at the end of 7 weeks, and then a gradual decrease. In this group, the animals survived for approximately 10 weeks after the transplantation of the tumor. In the other experiment (Table III, Grp. II) the growth of the tumor was slower, and the animals survived for 14 weeks. Furthermore, the increase in nucleic acid content was not of the same magnitude. It follows from these data that the nucleic acid changes are related to the size of the tumor and to its rate of growth.

Similar changes in the nucleic acid content were observed in the tissues of animals bearing spontaneous mammary tumors(18). The data obtained in 2 groups of 5 females of the Swiss strain which were 10 months old and 2 groups of 5 litter mate controls without tumors are shown in Table IV. They show that the liver nucleic acid content is lower than that found in the controls in the other experiments. This may be due to the fact that these animals were older. In the experimental animals, of the tissues studied, the lung showed a significant rise in its nucleic acid content. If the ratio RNA/DNA in the tumor is calculated from the values given in Tables I, II and III, it will be found that it generally falls as the tumor growth progresses. Table V shows the nucleic acid, purine, and pyrimidine content of melanoma S-91. It may be seen from these data that both RNA and DNA values are lower in the necrotic portion as compared to the non-necrotic portion of the same tumor. The values for purines and

TABLE IV. Nucleic Acid Changes in the Tissues of Swiss Mice Bearing Spontaneous Mammary Tumors in mg/g Dry Wt.

Tissue	Controls		Experimental	
	RNA	DNA	RNA	DNA
Liver	18.8; 22.2	5.3; 6.4	20 ; 21.2	8.8; 9.1
Kidney	17.8; 18	12 ; 13.7	18.6; 23.4	18.6; 21.8
Lung	12.4; 12	15.9; 16.9	15.7; 16.7	32 ; 30.6
Spleen	31.2; 29.7	95.6; 116.7	44.7; 39.2	98.8; 100.4

TABLE V. Nucleic Acid, Purine and Pyrimidine Content of Melanoma S91.

Age of tumor, wk	No. of tumors studied	RNA	DNA	Guanine	Adenine	Uracil	Thymine	Cytosine
		mg/g dry wt				μg/100 mg dry wt		
4	5	56	48	13.2	12.3	—	—	—
5	5	57.2	44.5	12.35	9.5	—	—	—
6	5	54.3	44.2	11.69	9.09	245	588	1052
7	4	49.7	34.4	12.02	9.63	243	433	946
10	4	50.2	54.9	13.12	9.65	178	573	953
*		55.4	61.6	13.50	10.46	245	626	1137
†		43.3	52.3	11.78	7.92	159	439	851
12	4	43	52.3	10.10	8	193	533	935
*		51.8	61.7	10.45	8.20	257	599	1018
†		39.2	45.2	8.78	7.26	155	482	793

* Non-necrotic portion. † Necrotic portion.

pyrimidines also indicate a similar trend. The drop in the ratio RNA/DNA may therefore be attributed to a decreased concentration of RNA in both the necrotic and the non-necrotic portions of the tumor, while DNA content decreases only in the necrotic portion.

Discussion. Reddy and Cerecedo(4) observed an increase in the nucleic acid content of the liver, lung, and kidney in mice during gestation. In the present paper it is shown that changes in the nucleic acids are also induced by neoplastic growth. These changes, however, are more pronounced than those found during gestation. Furthermore, the extent of these changes appears to be related to the rate of growth of the tumor. The observations of Greenstein and Andervont (15) in connection with liver catalase are of particular interest in this regard. They found that the decrease in liver catalase activity in mice was progressive with the growth of the tumor, and that the drop was most pronounced in animals bearing the most rapidly growing tumors. It is worthy of note that, in contrast to the effect of pregnancy on the nucleic acid content of various tissues observed in the mouse, embryonic tissue has no effect on the catalase activity of the liver(16). The work of Daoust and Cantero(17) is also in harmony with our findings. They found an increased activity of ribonucleopolymerase in the liver of the rat during the precancerous stage, and this increase was related to the RNA content of this organ.

Summary. 1. The nucleic acid content of various tissues has been determined in mice of the Swiss and dba strains bearing transplanted sarcoma 180 (Crocker), in dba mice bearing malignant melanoma S-91, and in Swiss mice bearing spontaneous mammary tumors. 2. In the mice with transplanted tumors, an increase in the pentose nucleic acid (RNA) and desoxypentose nucleic acid (DNA) content of the liver and lung was observed, whereas the kidneys showed a similar rise in the DNA. In the mice with mammary tumors, the most marked increase was found in the lung nucleic acids. 3. The changes of the nucleic acids in the dba mice could be related to the size and the rate of growth of the tumor. 4. The ratio RNA/DNA in the tumor usually dropped with the growth of the tumor. This drop is attributed to a decreased concentration of RNA in both the necrotic and non-necrotic portions of the tumor, whereas the DNA content dropped only in the necrotic portion. 5. The nucleic

acids, purines, and pyrimidines in malignant melanoma S-91 have been determined at various stages of its development. The lower values for uracil and thymine found in the necrotic portion of the tumor as compared with those found in the non-necrotic portion point also to a drop of both RNA and DNA in the necrotic portion.

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ACTH and Cortisone Aggravation or Suppression of the Febrile Response of Rabbits to Bacterial Endotoxin. (19182)

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Recent reports(1,2) indicate that ACTH and cortisone reduce the febrile response of rabbits to typhoid vaccine and, furthermore, cortisone has been shown to markedly reduce the temperature of patients with typhoid fever(3). Since the somatic antigens of Salmonella and Shigella organisms account for practically all of the toxicity of these bacteria, and may be responsible for the febrile reactions accompanying infections produced by these organisms(4-6), it was decided to investigate the effects of ACTH and cortisone on the response of rabbits to intravenous injections of a bacterial endotoxin, the purified somatic antigen of *Shigella dysenteriae* (*Shiga*)(7). Since some recent experiments suggested that ACTH and cortisone had no effect on the febrile response of rabbits to crude endotoxin prepared from *S. typhosa*(8) in contrast to the results reported by the other investigators(1,2), special attention was paid in these studies to an analysis of the time-dose relationship of the bacterial endotoxin and the ACTH or cortisone since the variations in effect produced could be due to differences in dosage and time of injection of the hormones.

Method. Young adult, male, albino rabbits weighing from 2.5 to 3 kg were given preparatory treatment with ACTH and cortisone* in groups of 2 animals each. One

* The ACTH was supplied through the kindness of Armour and Co. and the cortisone by Merck and Co.