

The Effect of Sarcoma 180 and Other Stressing Agents upon Adrenal Adenine Nucleotide-metabolizing Enzymes*

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SUMMARY

The effects of Sarcoma 180, heterologous tissue implantation, and turpentine treatment upon adrenal and liver adenine nucleotide-metabolizing enzymes were investigated. Adrenal 5'-nucleotidase activity was elevated significantly by progressive S-180 growth. No definite alteration in adrenal 5'-nucleotidase activity was induced by heterologous tissue implantation. Turpentine treatment depressed adrenal 5'-nucleotidase activity. Liver 5'-nucleotidase activity was elevated by tumor growth but not altered by the other stresses studied.

Sarcoma 180 caused a consistent increase in adrenal ATPase activity as compared with the daily control values. Heterologous tissue implantation and turpentine-induced stress did not affect adrenal ATPase levels. Liver ATPase activity was not significantly altered by any of the experimental conditions.

ACTH treatment caused an increase in adrenal 5'-nucleotidase activity, correlating with the increased plasma corticosterone levels measured. ACTH treatment caused an initial decrease in ATPase activity, with subsequent elevation of enzyme activity after 6 days of treatment. The relationship of these enzymes to adrenal function and the differences in response to various stress conditions are discussed.

Studies are being conducted in this laboratory concerning the effect of progressive tumor growth upon adrenal function of the host. In an earlier report, we found that adrenal function, as measured by corticosterone content and corticosterone levels in plasma, varied during different stages of Sarcoma 180 growth in Swiss mice (6). Nonspecific stressing agents induced changes in adrenal function which were similar to the changes observed in the tumor-bearing animals (6). These results stimulated further experimentation to investigate the effect of various stresses upon other aspects of adrenal metabolism.

This report is concerned with the effect of several stresses upon two adenine nucleotide-metabolizing enzymes, adenosine triphosphatase (ATPase) and 5'-nucleotidase. ATPase was se-

lected as one of the enzymes to be investigated, because it has been reported that this enzyme is a measure of tissue function (8). The selection of the other phosphatase, 5'-nucleotidase, was based on our studies suggesting that this enzyme was involved in the action of ACTH upon the adrenal cortex (7).

Since certain trends with time in regard to adrenal weight and corticosterone levels have been previously noted in the tumor-bearing host, it was necessary to perform daily enzyme assays on adrenal glands. The liver, an organ which is influenced by adrenal function, was also removed from the same animals, and enzyme assays were performed. The data presented here demonstrate that the activities of adrenal ATPase and 5'-nucleotidase and of liver 5'-nucleotidase are increased in animals bearing Sarcoma 180.

In addition to the tumor studies, normal (tumor-free) animals were exposed to nonspecific stressing agents. Adrenal glands and livers from these animals were removed at daily intervals and assayed for ATPase and 5'-nucleotidase activities. These data indicate that the nonspecific

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stresses studied here produce different effects upon these two enzymes when compared with the enzyme changes induced by progressive tumor growth. The effect of daily injections of ACTH upon these two adrenal enzymes was also studied and is reported.

MATERIALS AND METHODS

Sarcoma 180 was transplanted subcutaneously into the inguinal region by trocar technic. Female Swiss mice, weighing 18–22 gm., were used. Tumor-free animals served as controls. All animals were fed laboratory chow ad libitum and had access to water at all times. Animals were sacrificed by cervical dislocation at various intervals, six animals per group. Tumors, adrenals, and livers were removed immediately and weighed. Tissues for subsequent enzyme assays were kept iced until homogenization. Because of the small amount of tissues, the glands from each group were pooled prior to homogenization. Tissue pools were homogenized in conical, ground-glass homogenizers with ice-cold deionized water used as diluent.

Studies were performed with other types of stressing agents. A series of animals were implanted by trocar technic with rat femur muscle, of a size similar to that of the tumor implants. Another series of animals was stressed by daily subcutaneous injection of 0.05 ml. of turpentine, control animals receiving daily injections of 0.05 ml. of menstruum.¹ Animals were sacrificed by cervical dislocation at various intervals, and adrenals and livers were removed and assayed for enzyme activity as described below.

ATPase activity was assayed by the procedure of DuBois and Potter (2), modified by use of 2 per cent tissue homogenates. The ATPase incubation mixture contained, in a final volume of 0.65 ml., 7.5 mmoles sodium barbital buffer, pH 7.4; 2 mmoles each of CaCl₂ and ATP (disodium salt, Pabst Biochemical); and 0.1 or 0.2 ml. of tissue homogenate. 5'-Nucleotidase was assayed by a modification of the method of Cochran *et al.* (1), with a 2 per cent homogenate used for adrenals and livers. The incubation mixture contained 3.75 mmoles sodium barbital buffer, pH 8.9; 2 mmoles each of MgCl₂ and 5'-AMP (muscle adenylic acid, Pabst Biochemical); and 0.1 or 0.2 ml. of tissue homogenate, all components in a final volume of 0.65 ml. The procedure was further modified by using a 1-hour incubation at 38° C. The details

¹ The menstruum is an aqueous solution containing 0.4 per cent Tween 80, 0.7 per cent sodium chloride, 0.9 per cent benzyl alcohol, and 0.5 per cent carboxymethyl cellulose.

concerning the kinetics and stoichiometry of this reaction have been reported elsewhere (7).

The enzyme reactions were stopped by the addition of 0.1 ml. of 50 per cent trichloroacetic acid. After centrifugation, inorganic orthophosphate (P_i) was measured in the supernatant by a modification of the Fiske-Subbarow procedure (3). Nitrogen determinations were performed in duplicate on aliquots of the original homogenate by a modification of the Pregl Micro-Kjeldahl method (9). Enzyme activity was calculated as μ moles P_i liberated/mg tissue nitrogen. Each value represents the mean of duplicate assays on the pooled homogenate, and the data are presented by plotting the linear regression line of enzyme activity versus days on experimentation. The numerical mean was obtained of enzyme activities from the daily pooled samples of the control animals, each sample representing six normal animals, and the standard error was calculated. This standard error was of the same order of magnitude as reported earlier for the corticosterone determination (6).

RESULTS

The rate of growth of Sarcoma 180 was similar to that reported earlier (6), the tumor becoming palpable 3 days after implantation. The tumor-bearing animals survived 18–20 days following implantation. The changes in the thymus and adrenal weights during tumor growth were also similar to the data previously reported—namely, a decrease in thymus weight as tumor growth progressed and an early increase in adrenal weight followed by a decrease.

The nitrogen contents of adrenals and livers are recorded in Table 1. These values, which represent a mean of all the determinations, indicate no differences between normal (control) and tumor-bearing animals. Data are also presented for the nitrogen content of adrenals and livers from animals with rat femoral muscle implants and animals treated with turpentine. Turpentine treatment appeared to increase the nitrogen content of the adrenals. No other differences were observed when compared with the control animals.

Effect of stress upon adrenal ATPase activity.—The effects of Sarcoma 180, rat femoral muscle implantation, and turpentine treatment upon adrenal ATPase activity are shown in Chart 1. The slope of the linear regression curve of ATPase activity in the adrenals of the tumor-bearing animals was not statistically significant. This was interpreted as meaning that no correlation could be made between adrenal ATPase activity and the length of time following tumor implantation. However,

adrenal ATPase activity appeared to be consistently elevated in the tumor-bearing host. The numerical mean calculated from the adrenal ATPase activity of the tumor-bearing animals ($26.7 \pm 1.3 \mu\text{moles } P_i/\text{mg N}$) was significantly higher ($P < 0.01$) than the mean adrenal ATPase activity of the control animals ($19.7 \pm 1.0 \mu\text{moles } P_i/\text{mg N}$). Neither of the two nonspecific stresses studied, rat femoral muscle implantation and turpentine injection, altered the adrenal ATPase activity.

Effect of stress upon adrenal 5'-nucleotidase activity.—Chart 2 illustrates the effect of the various stresses studied upon adrenal 5'-nucleotidase activity. Progressive tumor growth induced an increase in adrenal 5'-nucleotidase activity, produc-

were assayed for alterations in ATPase and 5'-nucleotidase activities. No differences were observed in liver ATPase activity during the experimental period studied with the three experimental stresses reported here. However, liver 5'-nucleotidase activity appeared to be affected by tumor growth. The data, as shown in Chart 3, illustrate

TABLE 1
NITROGEN CONTENT OF ADRENALS AND LIVERS OF SWISS MICE

TREATMENT	NITROGEN ($\mu\text{G}/\text{MG TISSUE}$)	
	Adrenal	Liver
Control	$20.4 \pm 0.6^*$ (17.8–26.2)†	32.8 ± 0.5 (30.6–34.4)
Sarcoma 180	19.3 ± 0.5 (15.7–22.8)	30.8 ± 1.5 (28.0–33.9)
Rat femoral muscle implant	22.2 ± 0.7 (17.3–25.5)	32.2 ± 1.1 (28.0–40.8)
Turpentine	25.0 ± 0.9 (20.5–28.9)	31.1 ± 1.0 (27.7–38.7)

* Values represent the numerical mean of all nitrogen determinations performed on the daily pooled homogenates. The means are presented with the standard error of the mean.

† Values in parentheses are the range of the nitrogen determinations obtained during the experimental period studied.

ing a linear regression curve with a highly significant slope ($P < 0.01$). Toward the end of the experimental period, the adrenal 5'-nucleotidase activity in tumor-bearing animals was 2–3 times greater than that in the control animals. Rat femoral muscle implantation did not cause any change in adrenal 5'-nucleotidase activity, as can be seen from the data in Chart 2. The stress induced by daily injections of turpentine appeared to cause a decrease in adrenal 5'-nucleotidase activity, since the 5'-nucleotidase activity in the turpentine-treated animals was always lower than the 5'-nucleotidase activity in the control animals sacrificed at the same time.

Effect of stress upon liver ATPase and 5'-nucleotidase activities.—Livers of these same animals

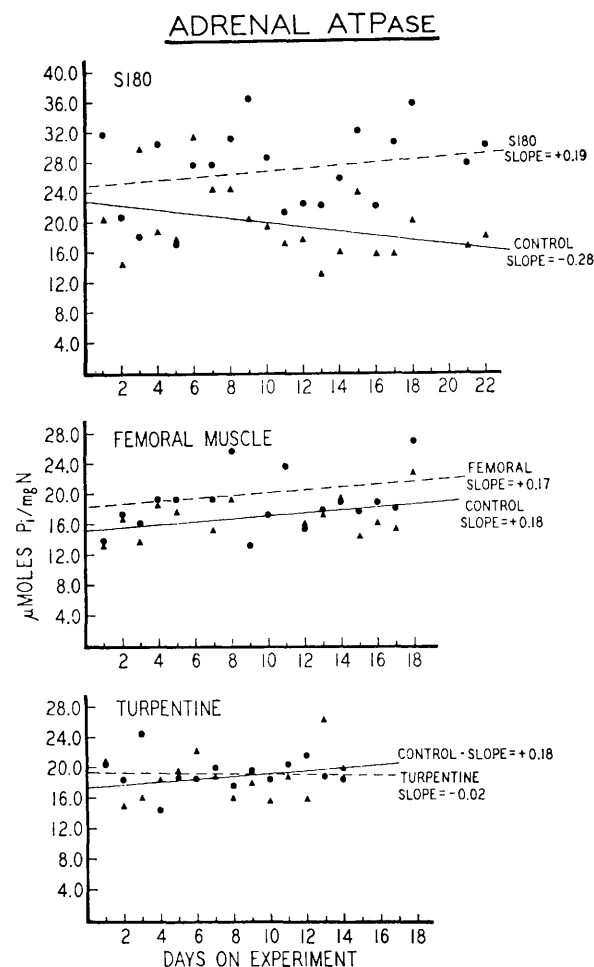


CHART 1.—The effects of Sarcoma 180, rat femoral muscle implantation, and turpentine treatment upon adrenal ATPase activity. The data are presented as linear regression lines, the ATPase activity of the control animals (\blacktriangle) and of the experimental animals (\bullet) being plotted against days on experiment. Each point represents the mean of duplicate enzyme assays performed on the pooled homogenate of tissues obtained from six animals.

that liver 5'-nucleotidase activity increased as tumor growth progressed. The change was similar to that observed in adrenal 5'-nucleotidase activity in the tumor-bearing host. Although the actual values fluctuate from day to day, the liver 5'-nucleotidase activity was always higher in the

tumor-bearing host after 7 days of tumor growth. Neither of the other stresses studied, heterologous tissue implantation and turpentine treatment, caused changes in liver 5'-nucleotidase activity which could be correlated with time.

Effect of ACTH stimulation upon adrenal ATPase and 5'-nucleotidase activities.—In an attempt to correlate adrenal function with the enzyme changes which were observed during tumor growth and other stress conditions, we decided to study the effect of continuous ACTH stimulation upon adrenal adenine-metabolizing enzymes.

It was felt that the enzyme changes induced by ACTH treatment would offer a basis of comparison between direct and specific adrenal stimulation and nonspecific adrenal activation (e.g., tumor growth).

ACTH (100 milliunits) was injected daily into normal mice, and the adrenal ATPase and 5'-nucleotidase activities were assayed from animals sacrificed daily. Blood was also collected from these same animals, and plasma corticosterone levels were determined fluorometrically. All determinations

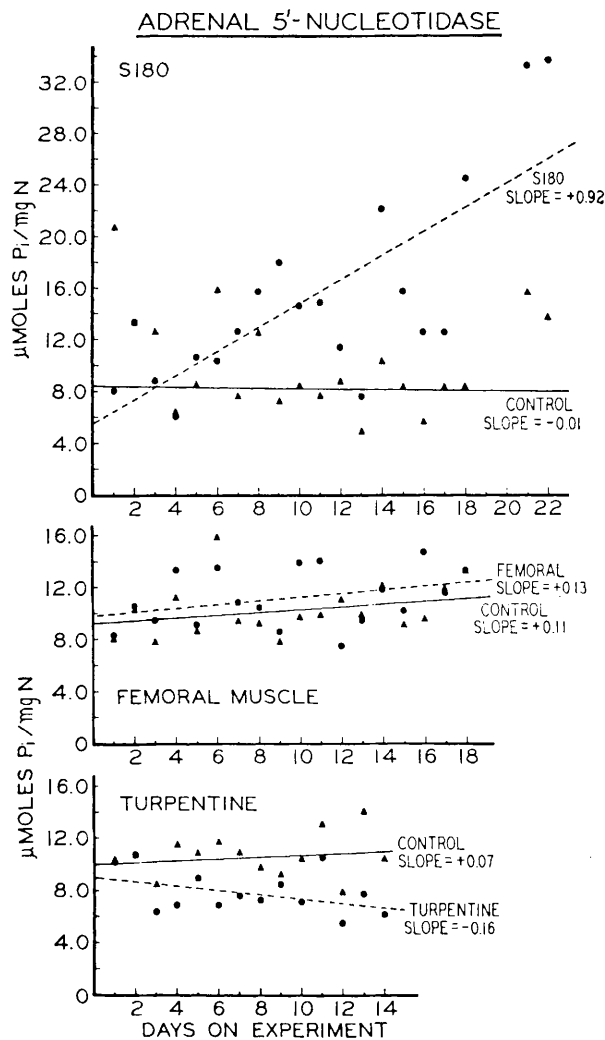


CHART 2.—The effects of Sarcoma 180, rat femoral muscle implantation, and turpentine treatment upon adrenal 5'-nucleotidase activity. The data are presented as linear regression lines, the 5'-nucleotidase activity of the control animals (▲) and the experimental animals (●) being plotted against days on experiment. Each point represents the mean of duplicate enzyme assays performed on the pooled homogenate of tissues obtained from six animals.

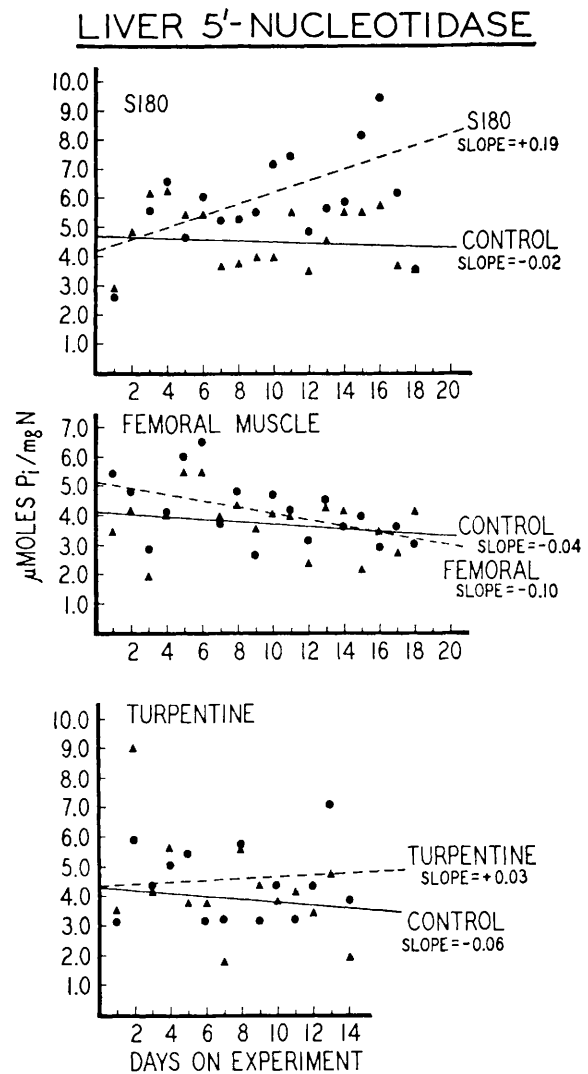


CHART 3.—The effects of Sarcoma 180, rat femoral muscle implantation, and turpentine treatment upon liver 5'-nucleotidase activity. The data are presented as linear regression lines, the 5'-nucleotidase activity of the control animals (▲) and the experimental animals (●) being plotted against days on experiment. Each point represents the mean of duplicate enzyme assays performed on the pooled homogenate of tissues obtained from six animals.

were also performed upon adrenals and plasma obtained from control animals. The control animals received daily injections of sterile menstruum without ACTH, in an attempt to simulate the effect of handling, restraining, and injection of the animal. The results, which are recorded in Table 2, are compared with the results obtained from the control animals sacrificed at the same time. Daily injections of ACTH caused an increase in adrenal 5'-nucleotidase activity as well as the expected increased plasma corticosterone levels. An apparent initial decrease in adrenal ATPase activity, as compared with that in the daily control animals, was observed during ACTH treatment. However, an indication of an elevation in adrenal ATPase was noted toward the end of the period studied, as

the tumor-induced adrenal 5'-nucleotidase changes were not due entirely to the fact that S-180 is not an isologous tumor for the Swiss mouse. It should be noted that excision of the rat femoral muscle implant site revealed connective tissue encapsulation of the implant, little or no vascularization in the immediate area of the implant, and no change in weight of the implanted tissue.

Turpentine-induced stress produced quite a different effect upon adrenal 5'-nucleotidase activity. Daily turpentine injections appeared to depress adrenal 5'-nucleotidase activity, the treated animals' 5'-nucleotidase activity being consistently lower than that of the control animals.

The exact relationship of 5'-nucleotidase and adrenal function is not known. We have reported

TABLE 2
EFFECT OF DAILY INJECTIONS OF 100 M.U. ACTH UPON ADRENAL 5'-NUCLEOTIDASE
AND ATPASE ACTIVITIES AND PLASMA CORTICOSTERONE LEVELS

DAYS OF TREATMENT	ADRENAL 5'-NUCLEOTIDASE ACTIVITY*			ADRENAL ATPASE ACTIVITY†			PLASMA CORTICOSTERONE LEVELS‡		
	Control	ACTH	% Change	Control	ACTH	% Change	Control	ACTH	% Change
1	5.2§	5.5	6.3	15.8	18.1	14.3	0.249	0.695	179.1
2	4.2	6.1	46.2	14.2	11.0	-22.7	0.270	0.675	150.0
3				12.6	11.0	-12.8	0.260	0.956	267.7
4	7.4	12.9	73.9	20.6	12.3	-40.6	0.342	0.557	62.9
5	5.5	9.7	76.5	13.2	11.9	-9.8	0.312	0.510	63.5
6	3.2	5.5	70.0	7.7	14.8	91.7	0.240	0.498	107.5
7	3.9	4.2	8.3				0.218	0.840	285.3
8	4.2	6.8	61.5	12.3	26.1	113.2	0.240	0.259	7.9

* Activity expressed as μ moles P_i /mg nitrogen/60 minutes at 38° C.

† Activity expressed as μ moles P_i /mg nitrogen/15 minutes at 38° C.

‡ Plasma corticosterone levels are in terms of μ g/ml.

§ Each value represents the mean of duplicate determinations on the pooled homogenate from six animals.

best illustrated by the per cent change from the values obtained for the control animals sacrificed simultaneously.

DISCUSSION

The findings presented in this report indicate that the activities of the adenine nucleotide-metabolizing enzymes studied are altered by the stresses studied as well as by specific adrenal stimulation resulting from ACTH treatment.

As illustrated in the results, adrenal 5'-nucleotidase appeared to be affected in a different manner by each of the experimental stress conditions studied. Sarcoma 180 caused a progressive increase in the activity of this enzyme in the adrenal. Rat femoral muscle implantation did not produce an alteration of adrenal 5'-nucleotidase activity. This lack of response when heterologous tissue implantation was used, as compared with the results obtained during S-180 growth, would indicate that

that adrenal 5'-nucleotidase activity increased within 30 minutes following treatment with a single dose of ACTH (7). These data, along with other studies, suggested that this enzyme is involved in the metabolism of adenosine 3',5'-monophosphate, a nucleotide implicated by Haynes *et al.* (4, 5) in the mechanism of ACTH stimulation upon the adrenal gland. Further evidence that 5'-nucleotidase is affected by ACTH treatment is presented here, as shown by the elevated adrenal 5'-nucleotidase activity during continuous ACTH treatment. If adrenal 5'-nucleotidase activity does reflect some aspect of adrenal function, then it may be concluded that progressive tumor growth causes a continuous stimulation of the adrenal gland of the host. These results are unique when compared with the effects of heterologous tissue implantation or turpentine treatment.

An effect of Sarcoma 180 upon adrenal ATPase was also noted. The lack of significant slopes of

the linear regression curves of adrenal ATPase activity indicated that no correlation could be made between elevated ATPase activity and time following tumor implantation. However, comparison of the mean adrenal ATPase activity of the tumor-bearing host with the control animals revealed a significant over-all increase in the adrenals of animals bearing Sarcoma 180. Neither heterologous tissue implantation nor turpentine treatment had a significant effect upon adrenal ATPase activity regardless of the statistical analysis made. At present, it is difficult to interpret the meaning of alterations of ATPase activity. It has been suggested by Potter *et al.* (8) that ATPase activity is a reflection of tissue function, and one might interpret the consistently elevated adrenal ATPase activity in the presence of a growing tumor as reflecting increased adrenal function. However, this interpretation does not appear to explain the initial decrease in adrenal ATPase activity following ACTH treatment. Further work will be necessary to clarify this discrepancy.

In contrast to the results obtained in the adrenal glands, liver ATPase was not affected by the three experimental conditions studied here. However, liver 5'-nucleotidase levels were increased by tumor growth. This again suggests that the liver may be involved in the metabolism of cyclic, adenosine 3',5'-monophosphate. The involvement of the liver, either directly or indirectly as influenced by adrenal function, might be a result of the role that this cyclic nucleotide plays in stimulating glycogenolysis by activation of phosphorylase A (10).

Finally, it should be pointed out that the effects of various stress conditions upon the enzymes studied should be viewed as an over-all pattern of

alteration during the entire time period studied. The data show that there are variations in enzyme activity in the control animals, a result which can be anticipated when dealing with such a dynamic organ as the adrenal. However, it was felt that investigation at less frequent intervals could lead to erroneous conclusions.

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