hyodeoxycholic acids, which bring about striking changes in hepatic cholesterol levels and synthesis rates in the mouse, but have little or no effect on small intestine or kidney. It would appear that in the mouse or rat, homeostatic control of cholesterol metabolism in kidney and intestine is more or less independent of hepatic control and of the bile acids influencing cholesterol synthesis in the liver.

Summary. Feeding various bile acids to mice for 3 weeks had the following effects: 1. Cholic acid increased hepatic and intestinal cholesterol levels, but had no effect on kidney cholesterol. It decreased hepatic cholesterol synthesis rates, but had no effect on intestinal synthesis rates. 2. Hyodeoxycholic and lithocholic acids decreased liver cholesterol levels. Hyodeoxycholic acid had no effect on intestine and kidney cholesterol, or on intestinal cholesterol-x-C^{14}. Both acids effected large increases in hepatic cholesterol synthesis. 3. Deoxycholic acid significantly decreased liver, small intestine and kidney cholesterol levels. It also decreased hepatic cholesterol synthesis. 4. Results of this study suggest that homeostatic control of cholesterol metabolism in tissues other than liver is independent of hepatic control.

1. Beher, W. T., Baker, G. D., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 892.
2. Beher, W. T., Baker, G. D., Anthony, W. L., *ibid.,* 1959, v100, 3.
3. Beher, W. T., Baker, G. D., *ibid.,* 1959, v101, 214.
4. Pihl, A., *Acta Physiol. Scand.,* 1955, v34, 206.
5. Bergstrom, S., Danielsson, H., *ibid.,* 1958, v43, 1.
6. Beher, W. T., Anthony, W. L., *J. Nutrition,* 1954, v52, 519.
7. ———, PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 356.

Received July 7, 1959. P.S.E.B.M., 1959, v102.

Relative Abundance of Vasopressin and Corticotrophin-Releasing Factor in Neurohypophysial Extracts.* (25233)

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In our previous experiments using rats with median eminence lesions to assay adrenocorticotrophin (ACTH)-releasing activity, the ACTH-releasing potency of posterior pituitary preparations was accounted for by their vasopressin content(1,2). Saffran and Schally (3) and later Guillemin and co-workers(4) reported, on the other hand, that extracts of posterior lobe of the pituitary gland contain a corticotrophin-releasing factor (CRF), distinct from vasopressin, which releases ACTH from pituitaries, *in vitro*. Attempts to demonstrate activity of this CRF of Guillemin *in vivo* were unsuccessful(1) using rats with hypothalamic lesions. Recently, Royce and Sayers(5) reported the presence of a CRF distinct from vasopressin in extracts of calf stalk-median eminence tissue, assaying the activity in rats with median eminence lesions. This observation prompted a reinvestigation of the ACTH-releasing activity of various brain extracts from hypothalamus and other regions, utilizing rats with lesions to assess ACTH-releasing activity.

Methods. Preparation of animals. Hypothalamic lesions were made in the median eminence of the tuber cinereum using methods previously reported(1) except that the rats were injected intramuscularly with 60,000 U crystalline procaine penicillin G at operation. Rats drinking at least 100 ml of water during the first 24 hours post-operatively were selected for the assay procedure. This assay of ACTH-releasing activity was performed 48 hours after placing the lesions, since at that time such rats have been shown to be unresponsive to a variety of non-specific stimuli (1-2). Proper selection of coordinates can

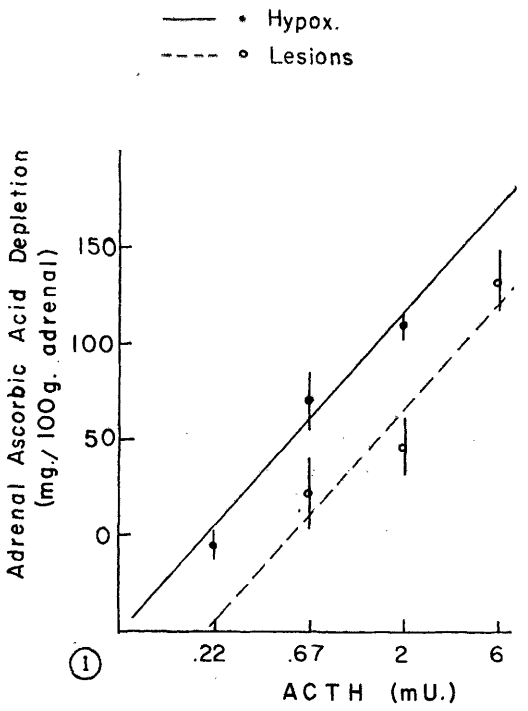
* Supported in part by Public Health Service Grants to S. M. McCann and Dr. Lewis Flexner.

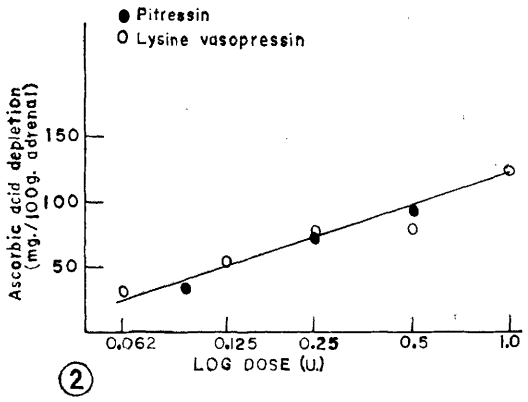
give nearly 100% successful operations using the above criteria. These animals are called rats with "acute" lesions. *Preparation of brain extracts.* Beef cortex and stalk-median eminence tissue were obtained at the slaughter house and placed in a beaker on dry ice. On arrival in the laboratory, the tissue from 20 brains was weighed, 40 ml of 0.2 M acetic acid were added, and the mixture was homogenized in a Waring blendor for 120 seconds. The mixture was refluxed for 10 minutes in a 100°C water bath and then centrifuged at 16,000 rpm for one hour in a Spinco ultracentrifuge(5). The supernatant was injected into assay animals. Rat stalk-median eminence tissue or brain stem was obtained from hypophysectomized rats, 24-48 hours after hypophysectomy, and ground in 0.5 ml 0.1 N HCl. One-tenth N HCl was added to pools of 6-12 stalk-median eminences to bring the volume to 0.5 ml per stalk-median eminence. After thorough mixing, the mixture was centrifuged at 3,000 rpm for 15 minutes, and the supernatant was injected into assay rats. *Bioassay for ACTH, ACTH-releasing, and pressor activity.* i) Assay for ACTH concentration of the extracts was performed in hypophysectomized rats,† 18-24 hours post-hypophysectomy, by the method of Sayers *et al.* (6) using USP reference standard corticotrophin as the standard. ii) ACTH-releasing activity was assessed in rats with "acute" lesions using adrenal ascorbic acid depletion as the index of ACTH activity(1). The left adrenal was removed under ether anesthesia to obtain a control value for adrenal ascorbic acid. One hour after intravenous injection of a test drug, the second gland was removed for analysis. iii.) Pressor activity of the extracts was measured in the urethane-anesthetized, dibenamine-treated rat as described by Dekanski (7). Statistical analysis was performed by standard methods for bioassays(8).

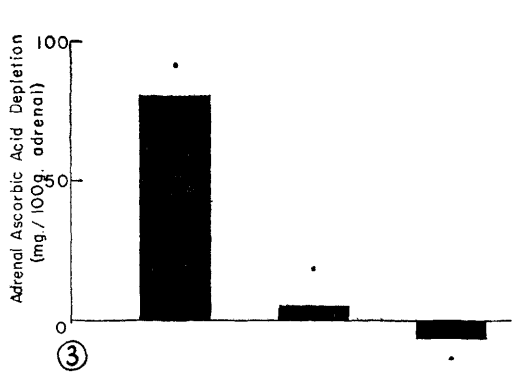
Results. Comparison of sensitivity to ACTH of hypophysectomized rats and those with "acute" lesions. Since ACTH content of the extracts of hypothalamic tissue was assessed in hypophysectomized rats, whereas ACTH-releasing activity was evaluated in rats with lesions, it was important to compare the sensitivity of these two types of rats to ACTH. The results of a multidose assay (Fig. 1) indicate that rats with lesions are 3 times less sensitive to ACTH than the hypophysectomized animals. Rats with lesions were of the Wistar strain while the hypophysectomized rats were Sprague-Dawleys. Consequently, the experiment was repeated using Sprague-Dawley rats with lesions to determine if a strain difference in sensitivity to ACTH was responsible for the results. Eight Sprague-Dawley rats with lesions also showed a diminished sensitivity to ACTH when tested at 2 dose levels. The difference in sensitivity to ACTH between hypophysectomized rats and those with lesions must be taken into consideration in evaluating the contribution made by ACTH to response to hypothalamic extracts in rats with lesions.

Activity of vasopressin in rats with "acute" lesions. To determine the contribution of vasopressin to activity of hypothalamic extracts, it was necessary to measure the ascorbic acid depletion resulting from injection of vasopressin in rats with "acute" lesions. The results obtained previously(2) with Pitressin and synthetic lysine-vasopressin are shown in Fig. 2. Responses to the 2 substances were similar when dose was expressed in pressor units. The ascorbic acid depletion resulting from administration of 0.1 U or 0.5 U of Pitressin (4 rats at each dose) in the present experiments did not differ significantly from that obtained previously. Consequently, these earlier results with lysine-vasopressin have been used to evaluate the contribution of vasopressin to the activity of the hypothalamic extracts. Royce and Sayers (9) reported that part of the activity of Pitressin in rats with "acute" lesions was due to an extra-pituitary action of vasopressin. This conclusion was based on their finding that Pitressin evoked ascorbic acid depletion in rats with "acute" lesions, which had been decapitated just prior to injection of the drug. Since our animals with lesions appear to be considerably less sensitive to vasopressin than theirs, it appeared of interest to test the effect of Pitressin in decapitated rats with "acute" lesions, as they had done, to determine if the

† Obtained from Hormone Assay Laboratories, Chicago, Ill.







ascorbic acid depletion induced by Pitressin could be accounted for by its effect in the decapitated rat having no pituitary gland. A dose of 0.5 U Pitressin, which induced an adrenal ascorbic acid depletion of 86 ± 13 mg % in 10 rats with "acute" lesions, produced a fall of only 30 ± 14 mg % in the ascorbic acid of 9 decapitated rats. The response of the decapitated rats was not significantly different from zero ($P<0.1$) and is significantly less than that found in rats with lesions ($P<0.01$). (The difference in response of the 2 types of rat is about the same as that reported by Royce and Sayers.) Failure to induce a significant depletion with Pitressin in the decapitated rat may have been related to the relatively low dose of Pitressin employed.

ACTH-releasing activity of beef stalk-median eminence extract (S.M.E.) This extract proved active in both the rat with lesions and the hypophysectomized rat (Table I). The minimal effective dose of 0.0032 of a single stalk-median eminence was derived from 0.5 mg wet weight of tissue. Ascorbic acid depletions obtained in the 2 types of assay animal were indistinguishable; however, since the rats with lesions were much less sensitive to ACTH than the hypophysectomized rats, statistical treatment of the data indicates that the ACTH content of the extract was 290 (167-502)‡ mU of ACTH/SME as assayed in the hypophysectomized rat, whereas the apparent ACTH potency in rats with lesions was 733 (440-1200)‡ mU ACTH/SME (Table III). Thus, ACTH contamination of the extract accounted for only 40% of its activity in rats with lesions and a significant, additional, ACTH-releasing activity was demonstrated.

FIG. 1. Comparison of sensitivity to ACTH of hypophysectomized rats and those with lesions. Vertical lines indicate stand. errors. Each point is the mean of 6 or 7 determinations.

FIG. 2. Comparison of activity of Pitressin and synthetic lysine-vasopressin in rats with lesions. Dosage is in pressor units. Reproduced from paper of McCann and Fruit(2).

FIG. 3. Adrenal ascorbic acid depletion induced by purified CRF of Royce and Sayers. Hypox. refers to hypophysectomized rats. Hydrocortisone refers to animals inj. with hydrocortisone. Dots above the bars indicate stand. errors.

‡ 95% confidence limits of the assay.

TABLE I. ACTH-Releasing Activity of Extracts of Beef Brain.

Extract	Dose, U*	Wet wt of tissue, mg	Pressor activity, mU	Adrenal ascorbic acid depletion, mean ± SEM (mg/100 g) Median eminence lesions	Hypophysectomized
Stalk-median eminence	.0125	2		168 ± 16 (5)‡	157 ± 17 (6)
	.0032	.5	13 (11-16)†	78 ± 27 (6)	66 ± 29 (5)
	.0016	.25		16 ± 19 (5)	
Cortex	1.0	200		111 ± 8 (4)	
	.1	20		14 ± 10 (5)	
	.0125	2		6 ± 14 (4)	

* 1 U is one stalk-median eminence or equivalent weight of cortex.
† 95% confidence limits.
‡ No. in parentheses is No. of rats in group.

The vasopressin content of 13(11-16)‡ mU at the minimal effective dose of extract could hardly have been responsible for this ACTH-releasing activity for the following reasons. Assuming that the pressor assay gives a valid estimate of the vasopressin content of the extracts, comparison of the responses to synthetic vasopressin and to beef extract in rats with lesions leads to the conclusion that only 5(2-10)‡ % of total activity of the extract can be due to vasopressin (Table III). Adding this 5% to the 40% accounted for by presence of ACTH leaves 55% of the activity presumably due to a CRF distinct from vasopressin.

An attempt was made to purify the active principle by selectively adsorbing the ACTH and vasopressin from this extract (and from a similar one obtained from calf tissue) with oxycellulose(5). Unfortunately, in our hands the oxycellulose almost quantitatively adsorbed all active material.

To determine the specificity of the responses to SME, an extract of cerebral cortex was prepared similarly and assayed in rats with lesions. The dose in units given in Table I represents a similar weight of tissue to that used to prepare SME. The dry weight of the extract was also comparable. No response was obtained until the dose was increased approximately 200 fold over that required for SME (Table I).

ACTH-releasing activity of stalk-median eminence extracts from hypophysectomized rats. SME from hypophysectomized rats was prepared and assayed in order to test extracts from another species and to eliminate ACTH contamination from the extracts. The results (Table II) indicate that one SME induced a significant ascorbic acid depletion in rats with lesions. Two SME's gave a marked depletion when no response was elicited in the hypophysectomized rat. Thirty-four mU of vasopressin were found per SME, indicating that 21(8-52)‡ % of the activity of this extract could be due to vasopressin. The remaining 79% was presumably accounted for by CRF (Table III). Extract prepared from an amount of the brain stem of hypophysectomized rats equivalent to the larger dose of stalk-median eminence was inactive in rats with lesions.

Corticotrophin-Releasing Factor of Royce and Sayers. An extract of calf stalk-median eminence purified by Royce and Sayers and

TABLE II. ACTH-Releasing Activity of Extracts of Rat Brain.

Extract	Dose, U*	Pressor activity, mU	Adrenal ascorbic acid depletion, mean ± SEM (mg/100 g) Median eminence lesions	Hypophysectomized
Stalk-median eminence	1	34 (26-43)†	40 ± 13 (9)‡	
	2		110 ± 5 (6)	14 ± 6 (6)
Brain stem	2		8 ± 11 (5)	

* 1 U is one stalk-median eminence.
† 95% confidence limits.
‡ No. in parentheses is No. of rats in group.

TABLE III. Summary of ACTH-Releasing Activity of Stalk-Median Eminence Extracts.

Extract	ACTH content (hypox. rats)	ACTH-like activity (lesion rats)	Vasopressin content	Estimate of % ACTH-like activity due to:		
	mU/SME			ACTH	CRF	Vasopressin
Beef	290 (167–502)*	733 (440–1200)*	4150 (3500–5000)*	40	55	5 (2–10)*
Rat, hypox.	≤ 0.2	3	34 (26–43)*	0	79	21 (8–52)*
Purified CRF (Royce and Sayers)	< "	7	37 (30–40)*	0	~96	~4

* 95% confidence limits.

kindly provided to us proved active in rats with lesions at a dose of 0.3 SME (Fig. 3). It was inactive in the hypophysectomized rat and contained only 11(9-12)‡ mU of vasopressin per 0.3 SME. Consequently, approximately 96% of the activity of this extract was attributable to CRF (Table III). This extract was inactive in the hydrocortisone-inhibited rat(10), but this absence of effect may have been due to the decreased sensitivity to ACTH of the steroid blocked rat since these rats gave a response of only 82 ± 10 to 6 mU of ACTH. This latter response is significantly less than that given by rats with lesions (133 ± 14; $P<0.02$).

Stability of ACTH-releasing activity. The activity of beef extract appeared to be quite stable in acid solution since refluxing for 10 minutes did not abolish it (see Methods), and since the extract was stable on storage at 4°C for 4 months. A dose of 0.0125 SME which initially gave an ascorbic acid depletion of 168 ± 16 (Table I) gave a response of 147 ± 7 when retested 4 months later in 5 rats. Extracts from rat hypothalamus likewise appeared to be stable at 4°C.

Location of lesions. In view of our wide experience with this particular lesion, study of the brains of these animals was restricted to gross examination, which indicated that the lesions were located in the basal hypothalamus in the region between optic chiasm and pituitary stalk. Serial sections of pituitaries from 8 rats revealed tissue which appeared normal and no infarcts were seen.

Discussion. In the present study rats with lesions in the median eminence were less sensitive to ACTH when tested 48 hours post-operatively than hypophysectomized rats tested 24 hours after operation. This is in contrast to the findings of Royce and Sayers that rats with lesions were at least as sensitive to ACTH as the hypophysectomized animal when tested under conditions apparently similar to those employed here(5). The reason for this difference is not apparent. The hypophysectomized rat is known to lose its sensitivity to ACTH under the conditions of the Sayers' assay with the passage of time, presumably because of absence of ACTH secretion(11). Similarly, the decreased output of ACTH in rats with lesions might have made these rats less sensitive than the hypophysectomized rats which were tested after a shorter post-operative interval. In a previous study from this laboratory(1), rats tested 48 hours after either lesions or hypophysectomy gave equivalent responses to a rather high dose of 5 mU of ACTH. This result is at least consistent with the suggestion that the decrease in ACTH sensitivity of rats with lesions in comparison with hypophysectomized rats tested in the present experiments may have been due to the greater time which had elapsed after operation in the rats with lesions. Other unknown causes may also have contributed to the diminished sensitivity of animals with lesions.

Since the rat with lesions is less sensitive to ACTH than the hypophysectomized rat, the question arises as to the role of decreased ACTH sensitivity in producing the block of ascorbic acid depletion in response to stress found in rats with lesions. Though decreased sensitivity to ACTH may be a factor in producing this failure of ascorbic acid depletion, the ascorbic acid depletion induced in normal rats by a stress such as unilateral adrenalectomy is of such a magnitude(12) as to suggest discharge of sufficient ACTH to deplete

TABLE IV. ACTH-Releasing Potency of Various Neurohypophysial Extracts.

Extract	% ACTH-releasing activity due to Vasopressin	CRF
Beef SME	9 (4- 18)*	91
Rat "	21 (8- 52)*	79
Protopituitrin†	141 (42-472)*	~ 0
Pitressin†	121 (46-319)*	~ 0
Purified CRF of Royce & Sayers	~4	~96

* 95% confidence limits.
† Calculated from data of McCann(1,2).

ascorbic acid even in the less sensitive rat with lesions. Failure of ascorbic acid depletion in rats with lesions would then be due both to reduced secretion of ACTH by such rats(13, 14) and to reduced sensitivity of the adrenal to the ACTH released.

The results presented here indicate that crude extracts from the stalk-median eminence region of beef and rat brain produce in rats with hypothalamic lesions a significant release of ACTH which cannot be accounted for by their content of vasopressin, thus, confirming the findings of Royce and Sayers(5). That this is a specific response to stalk-median eminence tissue is indicated by the lack of activity of extracts from beef cortex and rat brain stem when injected in comparable amounts. (The effect of beef cortex at a dose 200 times greater than that required with SME cannot be explained at this time. It may be a non-specific effect; on the other hand the lack of apparent toxicity of even this large dose of extract argues against this interpretation. A possible explanation is contamination of the extract with ACTH or CRF.)

The relative contribution of vasopressin and CRF to the ACTH-releasing activity of various neurohypophysial extracts is given in Table IV. No correction has been made for the relatively small proportion of the effect of vasopressin which can be accounted for by its action at extrahypophysial sites(9,15). It would appear that vasopressin can account for only 10-20% of the ACTH-releasing activity of the stalk-median eminence area, whereas it accounts for all of the activity found in posterior lobe extracts within the limits of error of the methods. The CRF found in posterior lobe extracts by Saffran and Schally(3) and by Guillemin and coworkers(4) would seem to be present in relatively small amounts. There appears then to be a differential localization of CRF and vasopressin in these 2 portions of the neurohypophysis.

Since lesions in the median eminence block ACTH release, whereas ACTH release can be induced in such animals by extracts from this same region containing vasopressin and CRF, it is concluded that vasopressin and CRF share the role of ACTH-releasing neurohumor. If storage in tissue is any criterion of secretion rates, it would appear that CRF is the principal ACTH-releasing substance from stalk-median eminence but that vasopressin is the chief factor in neural lobe. Secretion into hypophysial portal vessels is probably the critical factor in providing a sufficient concentration of neurohumor to the pars distalis. From this standpoint, CRF appears to be located in a more strategic position at site of origin of these vessels in the median eminence and stalk. However, vasopressin might reach the pars distalis in high concentration *via* the short portal vessels which arise in the neural lobe and drain blood to the pars distalis(16). Only further studies of the ACTH-releasing activity of portal vessel blood from these 2 sites and more information concerning the proportion of hypophysial blood flow coming from these 2 types of portal vessels can conclusively establish the relative importance of CRF and vasopressin for control of ACTH secretion.

Conclusions. Extracts of the stalk-median eminence area of beef or rat brain produce a specific release of ACTH in rats with hypothalamic lesions. The major portion of this effect is due to a corticotrophin-releasing factor. Ten to 20% of the activity of these extracts appears to be due to vasopressin.

1. McCann, S. M., *Endocrinol.,* 1957, v60, 664.
2. McCann, S. M., Fruit, A., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 566.
3. Saffran, M., Schally, A. V., *Endocrinol.,* 1955, v56, 523.
4. Guillemin, R., Hearn, W. R., Cheek, W. R., Householder, D. E., *ibid.,* 1957, v60, 488.
5. Royce, P. C., Sayers, G., PROC. SOC. EXP. BIOL.

AND MED., 1958, v98, 677.

6. Sayers, M. A., Sayers, G., Woodbury, L. A., *Endocrinol.,* 1948, 42, 379.

7. Dekanski, J., *Brit. J. Pharmacol.,* 1952, v7, 567.

8. Bliss, C. I., *The Statistics of Bioassay,* Academic Press, N. Y., 1952.

9. Royce, P. C., Sayers, G., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 70.

10. Porter, J. C., Jones, J. C., *Endocrinol.,* 1956, v58, 62.

11. Long, C. N. H., *Recent Prog. in Hormone Research,* 1947, v1, 99.

12. McCann, S. M., *Am. J. Physiol.,* 1953, v75, 13.

13. McCann, S. M., Sydnor, K. L., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 369.

14. Royce, P. C., Sayers, G., *Endocrinol.,* 1958, v63, 794.

15. Hume, D. M., in *Pathophysiologia Diencephalica,* ed. S. B. Curri and L. Martini, Springer, Wien, 1958, p225.

16. Daniel, P. M., Pritchard, M. M. L. *Quart. J. Exp. Physiol.,* 1956, v41, 215.

Received July 8, 1959. P.S.E.B.M., 1959, v102.

Effect of Temperature Extremes and Cortisone on Toxicity of Diphosphopyridine Nucleotide in Mice. (25234)

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It was reported recently(1) that analogs of nicotinamide cause toxic effects in mammals. Degenerative changes in the central nervous system appeared as prominent features of intoxication in mice and rats. Selective neuropathological damage was seen in nuclei of the brain stem and in gray matter of the anterior horn. Kaplan and coworkers(2) showed that analogs of nicotinamide participate in enzymatically catalysed exchange reactions with diphosphopyridine nucleotide (DPN), and they suggested that the toxicity of analogs is due to such reactions. These findings were confirmed and extended by Burton(3). Burton and others(4) could also demonstrate that reserpine and promazine prolong elevation of DPN concentration of tissues caused by injection of nicotinamide in mice. To examine DPN effects in a different manner, we determined first the toxicity of injected DPN. Then we investigated the effect of injected DPN on mice, as animals were subjected to stress of extreme environmental temperatures. Glock and McLean(5) found that starvation or administration of a variety of hormones alter DPN content of tissues considerably. It is well established that under stress of temperature extremes, especially cold, activity of the adrenal cortex increases significantly. Scherr reported(6) that toxicity of cortisone in mice varies according to environmental temperatures. After repeating his experiments we administered DPN and cortisone simultaneously, for evidence was offered by Munch *et al.*(7), that there is a specific interaction between steroid hormones and coenzymes like DPN.

Method. DPN was injected intraperitoneally to 20 Swiss mice, each weighing 24 g. The animals were observed behaviorally and histological studies of brain stem, muscles, kidneys and liver were performed.

Result. 105 mg of DPN/animal killed all mice in 4 hours. Before death, animals exhibit dysmetria, paralysis of hind legs, and marked general irritability. Neuropathological findings were similar to those described by Sternberg and Philips(1). Striped muscles, kidneys or liver showed no histopathological changes. Fifty mg of DPN is well tolerated intraperitoneally. Daily injection of this dose for 15 days, or more, produced no deleterious effects.

DPN injection and environmental temperatures. Method. Swiss mice, each weighing 24 g, in groups of 10, were exposed to hot environmental temperatures (37°C). Others, also in groups of 10, were subjected to cold environmental temperatures (7°C). They received 50 mg of DPN intraperitoneally. Control groups did not receive DPN.

Result. Mice exposed to environmental