Twenty μg of a known chelating agent, tetrasodium versenate, were added to the perfusate in place of chlortetracycline-HCl in 4 experiments. Cardiac irregularities developed which were almost identical with irregularities induced by chlortetracycline-HCl and which appeared in approximately the same time intervals (Fig. 3). In 3 experiments, the addition of 40 μg Mn^{++} as manganous chloride to the perfusate following onset of arrhythmia that had been induced by chlortetracycline alleviated the irregularities temporarily with resumption of a normal beat (Fig. 4).

Discussion. To account for the effect of chlortetracycline-HCl on the isolated frog heart, Yang and Harvey(5) proposed alteration of acetylcholine regulation of cardiac action as a possible explanation. If alteration of acetylcholine control resulted in accumulation of acetylcholine, the inhibitory effect of this mediator on heart rate would occur and inotropic and chronotropic irregularities would appear. Alteration of acetylcholine regulation of cardiac action might result from chelation of the co-factor of cardiac cholinesterase by chlortetracycline. Thus, cholinesterase would be unable to destroy acetylcholine which would accumulate and cause cardiac irregularity. Weidenheimer, Reed and Upham(4) demonstrated the ability of chlortetracycline to form complexes with 11 different metallic ions. Mn^{++} was not included in this list. However, Albert(1) showed that chlortetracycline will chelate Mn^{++} and Saz and Slie(3) found that Mn^{++} will reverse the inhibition of nitro reductase by chlortetracycline.

Summary. The effect of chlortetracycline-HCl on the isolated rat heart is practically identical with the effect of a known chelating agent, tetrasodium versenate. This effect can be reversed temporarily by Mn^{++}. Thus, chlortetracycline seems to cause irregularities in the isolated rat heart by chelation of the co-factor of cholinesterase which inactivates this enzyme and permits acetylcholine to accumulate and to exert its inhibitory effect on the heart. The co-factor of cardiac cholinesterase may be Mn^{++}.

1. Albert, Adrien, *Nature,* 1953, v172, 201.

2. Harvey, Paul A., and Yang, Wei, *Science,* 1953, v118, 752.

3. Saz, Arthur K., and Slie, Rita B., *J. Biol. Chem.,* 1954, v210, 407.

4. Weidenheimer, Joseph F., Reed, Charles G., and Upham, Sidney D., *Chem. Abst.,* 1953, v47, 8325.

5. Yang, Wei, and Harvey, Paul A., unpublished data.

Received July 18, 1956. P.S.E.B.M., 1956, v92.

Regulation of Heat Production in Cold-Adapted Rats.* (22632)

W. H. COTTLE AND L. D. CARLSON.

Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle.

Recent studies(1,2) of adaptive changes induced in small mammals by prolonged exposure to cold have indicated that there is a reduction of muscular activity after adaptation. This demonstration along with the observation that heat production in the cold is increased(3,4) suggests that a regulatory mechanism not dependent on excitation of skeletal muscle is effective after adaptation. Such control of metabolic heat production was postulated by Rubner(5) to account for increased heat production not accompanied by increased muscular activity.

Regulation not due to muscular activity has been postulated to be mediated through release of epinephrine from adrenal medulla (6,7). The increase in such thermogenesis on cold adaptation may be due either to greater release of epinephrine or to greater sensitivity of adapted animals to the hormone.

* This research was supported in part by U. S. Air Force under Contract No. AF 18(600)-1467, monitored by Alaskan Air Command, Arctic Aeromedical Laboratory.

To test capacity of non-shivering means of regulating heat production after adaptation to cold, the metabolic response to cold of cold-adapted rats whose muscular activity was blocked by curare, was compared to that of similar nonadapted rats. To evaluate the role of the adrenal medulla in this response a number of adrenodemedullated rats were subjected to similar tests.

Methods. Male Sprague-Dawley rats were kept in individual metal cages, fed Purina ground chow, and given water without restriction. Eight rats were placed in a cold room at 5°C ± 1° for approximately 60 days. Eight control animals were kept at 28°C ± 2°. The lights in the cold room were regulated to approximate the normal day-night conditions of the room in which the non-adapted animals lived. Body weights ranged from 170-220 g at the time the rats were placed in the cold and 300-400 g at the end of the experimental period.

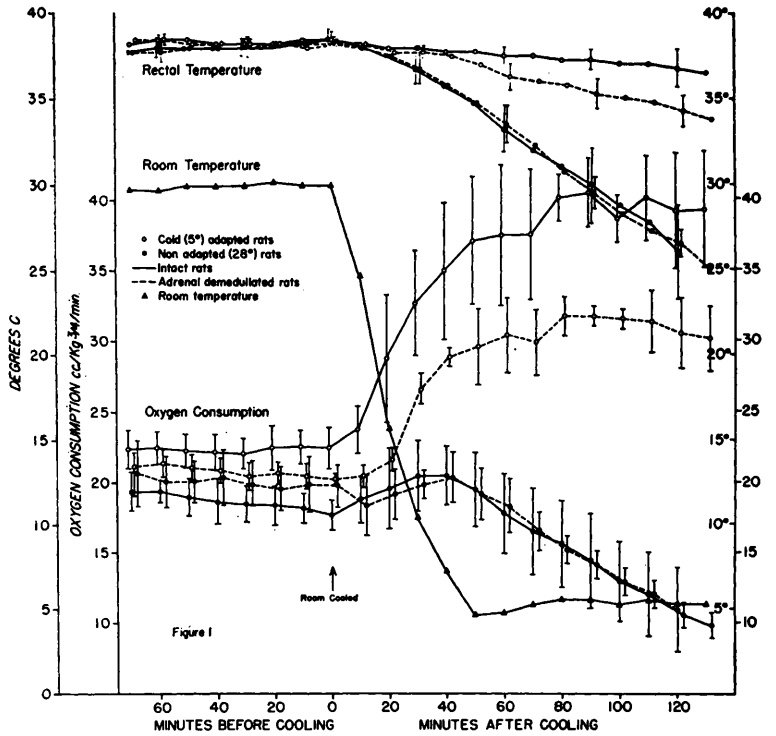
Thirty-five days after the animals had been placed in the cold, 4 were removed and along with 4 from the warm room were adrenodemedullated. Animals from the cold room were returned 8 hours after demedullation. Both groups were provided with 1% NaCl as drinking water for 10 days. At autopsy, the demedullated adrenals were removed and prepared for histological study.

At the end of the exposure period the metabolic response of each animal while curarized was tested separately. For this, the animal was prepared in a test room at 30°C. With the animal lightly etherized and supine on a board, the trachea was cannulated and the ventilatory pump connected. A dose of 360 μg of d-tubocurarine was then injected into the external jugular vein. Oxygen consumption was determined using an open circuit in which the expired air was passed through a Beckman Oxygen Analyzer and in which flow rate was determined by timing a sample of the expired air collected in a small spirometer. Output of the pump was regulated to maintain an expired oxygen tension of 120-130 mm Hg. Rectal, foot, and ambient air temperatures were measured with copper-constantan thermocouples recording on a Brown potentiometer. Electromyograms (EMG) and electrocardiograms (EKG) were recorded from needle electrodes inserted into muscles of the fore- and hindlimbs. When activity appeared in the EMG, an additional 180 μg tubocurarine were injected. For initial 90 minutes of the test (70 of which are indicated in Fig. 1) the room temperature was held at 30°C. It was then lowered to 5°C over a period of 30 to 40 minutes, and held at 5°C for an additional 100 minutes or until rectal temperature of the animal had dropped to 25°C. Throughout the test period, readings were made at 10-minute intervals. After the test each animal was rewarmed by radiant heat to its initial body temperature, and its oxygen consumption compared with that during the initial period to check the condition of the preparation.

Results. All rats adapted to 5°C were able to increase their heat production when exposed to cold even though muscular activity was blocked with curare (Fig. 1). In contrast, non-adapted animals were unable to increase heat production sufficiently to prevent a marked fall in rectal temperature.

The increase in heat production by the intact cold-adapted animals was sufficient to maintain rectal temperature at or near the initial level. Demedullated cold-adapted rats also increased their heat production but significantly less than that of the cold-adapted animals. Their rectal temperature fell. The response of the demedullated warm-adapted animals did not differ from that of the intact non-adapted. Histologically, the demedullated adrenals showed well-developed cortical tissue with the medulla replaced by scar tissue.

When the room temperature was 30°C, foot temperatures of the cold-adapted animals approximated the rectal temperatures, evidence of marked peripheral vasodilation. This is shown in Fig. 2 as a large difference between foot and ambient air temperatures. The foot temperatures of the nonadapted animals approximated ambient air temperature, evidence of peripheral vasoconstriction. As the room cooled, the temperature gradient from foot to air increased transiently, owing to different



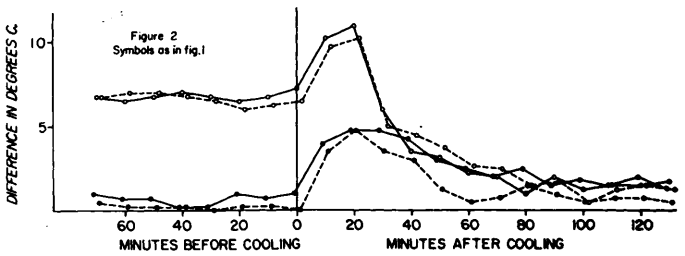


FIG. 1. Metabolic response of curarized rats to lowered ambient temperature. Mean oxygen consumption and rectal temperature of each group along with ambient temperature is plotted on ordinate; minutes before and after cooling on abscissa. Stand. dev. are indicated by vertical lines.

FIG. 2. Vasomotor responses of curarized rats to lowered ambient temperature. Temperature gradient between the feet of preparations and ambient temperature is plotted on ordinate; minutes before and after cooling on abscissa.

cooling rate of air and tissue and to delay in the onset of vasoconstriction. After cooling was completed, all animals showed marked vasoconstriction.

Discussion. The response observed indicates that the capacity of the non-shivering control of heat production is greater after adaptation to cold. This increase supports the suggestion(8) that this regulation of body heat is increased with adaptation to cold. Such a mechanism of augmenting metabolic heat production would have a sparing action on muscular activity and therefore would account for the reduction in the integrated electrical activity of skeletal muscle of rats(1,2) or the change in the electromyographic pat-

terns of rabbits(9) after adaptation to cold. It would also account for the quieter and more comfortable appearance of adapted animals in the cold(3) and for the ability of the adapted animals to maintain the high level of heat production enabling them to survive severe cold stress(10,11,12). The response of the cold-adapted adrenodemedullated rats indicates that the non-shivering regulation in the cold-adapted rat is mediated only in part by release of epinephrine from the adrenal medulla. If the balance of the response is produced by epinephrine then it must be due to epinephrine released by extra-adrenal medullary tissue or sympathetic endings for there was no evidence of regeneration of adrenal medullary tissue.†‡ In adrenodemedullated rats kept at room temperatures Vogt (13) and Bloom and Russel(14) found little or no increase in circulating epinephrine from conditions which caused its release in normal animals. The response of the cold-adapted demedullated rats to cold suggests that following adaptation more extra-adrenal epinephrine is produced either as a result of an increase in the amount secreted or a change in the ratio of norepinephrine to epinephrine produced. The response also may be to some extent a product of the increased sensitivity of cold-adapted rats to epinephrine. One plausible explanation of our results is suggested by the work of Ring(15) and of Swanson(16). The greater effectiveness of the non-shivering regulation after adaptation to cold could result from an increased sensitivity to the endogenous epinephrine which has been shown(6,7,17) to be released by animals on exposure to cold. The potentiation of the epinephrine response may be related to the increased thyroxine requirement of cold-adapted animals(18,19). The increase may be analogous to that seen in animals made hyperthyroid by administration of thyroxine (16,17).

Summary. (1). The capacity of cold-adapted and non-adapted rats to increase heat production by non-shivering regulatory means was tested. In both groups, muscular activity was blocked by curare. Only cold-adapted animals could increase heat production sufficiently to maintain body temperature as the test room was cooled. Adrenodemedullation reduced somewhat the ability of the cold-adapted rats to increase heat production, but made little change in the response of non-adapted animals. It is suggested that this regulation results from the release of adrenal and extra-adrenal epinephrine and that the increased heat production by cold-adapted animals results from an increased epinephrine sensitivity. (2). To our knowledge this is the first demonstration of non-shivering heat regulation of this magnitude. The response is augmented by adaptation to cold.

† Two acute spinal (C3) animals, 1 adapted and 1 nonadapted, cooled immediately when room temperature was lowered. Although the muscle in the heads of these animals showed shivering responses, oxygen consumption did not increase.

‡ Two acutely adrenalectomized cold-adapted rats also showed a marked response, suggesting that response of the demedullated animals did not result from compensatory changes.

1. Sellers, E. A., Scott, J. W., and Thomas, H., *Am. J. Physiol.*, 1954, v177, 372.

2. Heroux, O., Hart, J. S., and Decopas, F., *ibid.*, 1956, v183, 626.

3. Sellers, E. A., and You, S. S., *ibid.*, 1950, v163, 81.

4. Cottle, W., and Carlson, L. D., *ibid.*, 1954, v178, 305.

5. Rubner, M., *Die Gesetze des Energieverbrauchs bei der Ernahrung.* (Deuticke, Leipzig and Wien) 1902.

6. Cannon, W. B., Querido, A., Britton, S. W., and Bright, E. M., *Am. J. Physiol.*, 1927, v79, 466.

7. Morin, G., *Rev. canad. de biol.*, 1946, v5, 388.

8. Carlson, L. D., *Man in Cold Environment*, (U. S. Dept. of Commerce Office of Technical Services, Washington 25, D.C.) 1954.

9. ———, *Fed. Proc.*, 1955, v14, 26.

10. Sellers, E. A., Reichman, S., Thomas, N., and You, S. S., *Am. J. Physiol.*, 1951, v167, 651.

11. Blair, J. R., and Dimitroff, J. M., *Effect of Cold Acclimatization upon Resistance to Cold Injury in Rabbits and Rats.* (U. S. Army Medical Research Lab. Rept., No. 81, Ft. Knox, Ky.) 1952.

12. Hart, J. S., *Canad. J. Zool.*, 1953, v31, 80.

13. Vogt, M., *J. Physiol.*, 1952, v118, 588.

14. Bloom, W. L., and Russel, J. A., *Am. J. Physiol.*, 1955, v183, 356.

15. Ring, G. C., *ibid.*, 1942, v137, 582

16. Swanson, H. E., *Fed. Proc.*, 1956, v15, 183.

17. Hartman, F. A., McCardock, H. A., and Loder, M. M., *Am. J. Physiol.*, 1923, v64, 1.
18. Dempsey, E. W., and Astwood, E. B., *Endocrinology*, 1943, v32, 509.
19. Woods, R., and Carlson, L. D., *Endocrinology*, in press.

Received May 17, 1956. P.S.E.B.M., 1956, v92.

Effect of Fluorides and Other Solutions on Solubility of Powdered Enamel in Acid. (22633)

JOSEPH C. MUHLER.
Indiana University, Department of Chemistry, Bloomington.

Considerable interest has been shown recently in the sarcosinates and in dehydroacetate as possible inhibitory agents of human dental caries. It has been suggested that mechanism of action in this regard is the inhibition of acid formation in the mouth through interference with enzyme activity(1). However, a recent publication(2) suggests that the sarcosinates are as effective as fluorides in decreasing the solubility of powdered enamel in acid and, thus, may be of importance in prevention of dental caries by this mechanism also. Since stannous fluoride has been shown to be considerably more effective than sodium fluoride in reducing enamel solubility(3), experimental dental caries in rats(4) and hamsters(5), and human dental caries when applied topically(6) or in a dentifrice(7), it was of interest to compare sodium N-palmitoyl sarcosinate with stannous fluoride. Similarly, sodium oxalate(8) has been suggested as possessing "fluoride-like" protection action on enamel, and it was of interest to compare this compound with stannous fluoride and sodium N-palmitoyl sarcosinate.

Methods. The procedure used for measuring the effectiveness of the 5 compounds has been described(3,9). In this study, a 5.0-ml portion of an aqueous solution of the compound to be studied was added to a 100-mg portion of powdered enamel. In all instances the solutions were used as prepared without adjustment of the pH. After the mixture was shaken for 20 minutes at 25°C it was promptly centrifuged. The clear supernatant was decanted and 1.0 ml of redistilled fluoride-free water was used to wash the enamel. The supernatant and the wash solution were combined and suitable aliquots were analyzed for phosphorus(10). This served as a measure of the amount of decalcification occurring during the treatment period with any of the proposed protecting solutions. Following this, 10.0 ml of 0.2 N acetic acid (pH 4.0) was added to the powdered enamel, and the mixture was shaken for 20 minutes as before. Following prompt centrifugation the supernatant solution was decanted, the enamel was washed, and the combined solutions were analyzed for phosphorus. Controls were run in the same manner except that distilled water was used in place of the treatment solutions.

The amounts of phosphate dissolved by the treatment solution (fluoride, sarcosinate, dehydroacetate, or oxalate) and by the decalcification solution (acetic acid) were combined to give a figure representing the total amount of tooth substance lost. The effectiveness of each treatment solution was then calculated by comparing this value with the corresponding control value (for untreated enamel), and expressing the result as percentage reduction in solubility.

Results. The data obtained are presented in Table I. The stannous fluoride was by far the most effective compound tested; a 1% solution reduced enamel solubility by 86%, and a 0.1% solution was 85% effective. Under similar conditions, sodium fluoride reduced enamel solubility by 44 and 36%, respectively. The sodium N-palmitoyl sarcosinate was only moderately active, a 1.0% solution reducing the solubility of the powdered enamel by only 20% and a 0.1% solution by only 4%. Volker *et al.*(2) also evalu-