aminoazobenzene occurred. At the end of 30 hours, 90% of the p-dimethylaminoazobenzene initially added had disappeared and as much as 85% was accounted for in the form of the monomethyl derivative. Thereafter, the amount of p-monomethylaminoazobenzene decreased. Small amounts of p-aminoazobenzene also were formed. The demethylation occurred only during autoxidation; none was found when the oxidation was inhibited with tocopherol.

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Ergotoxine Hyper- and Hypothermia in Albino Rats.*

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In 1906 an alkaloid was isolated from ergot of rye by Barger and Carr[1] and called ergotoxine. A year later Barger and Dale[2] reported that "the high temperature at and after death seemed to be a characteristic effect of fatal doses of ergotoxine." Since these fundamental studies and throughout the succeeding 40 years, other alkaloids have been isolated and identified until at present we have to deal with 6 natural alkaloids of different composition, each with 2 isomers. Stoll and Hofmann[3] have shown that the compound called ergotoxine is not a uniform chemical substance, but a molecular complex of 3 alkaloids: ergocristine, ergocornine, and ergokryptine.

Sollmann,[4] in his discussion of the central nervous system effects of ergotoxine as shown by several investigators, stated that the body temperatures of rabbits are raised from 3 to 4.5° C by the intravenous injection of .5 to 1.5 mg per kg of ergotoxine. Since there was no rise in decapitated animals, it was assumed that the action must be central, and that it probably involved both increased heat production and diminished heat loss.

Goodman and Gilman,[5] on the other hand, considered the action of ergotoxine on the central nervous system, when administered in moderate amounts, to be variable and, as a rule, unimportant. They noted that large doses of ergotoxine, given intravenously in certain laboratory animals, produced marked excitement, sham rage, and "other evidence of stimulation of central sympathetic centers."

Githens[6] found that cats responded to ergotoxine by a rise of temperature, but that rats, mice and pigs exhibited falls in temperature. Youmans and Trimble[7] reported that ergotoxine produced practically no effect on body temperature in dogs. Rothlin,[8] in discussing the effect of the ergot alkaloids on temperature regulation, maintained that the action was of a central nature because it was suppressed by general anesthesia. Ergotamine, which according to Sollmann,[4] and Goodman and Gilman,[5] gives essentially the

* Supported by a grant from the Office of Naval Research.

1 Barger, G., and Carr, F. H., *J. Chem. Soc.* (London), 1907, **91**, 337.

2 Barger, G., and Dale, H. H., *Biochem. J.*, 1907, **2**, 240.

3 Stoll, A., and Hofmann, A., *Helv. chim. Acta.*, 1943, **26**, 1570.

4 Sollmann, T., *A Manual of Pharmacology*, 7th ed., W. B. Saunders Co., Philadelphia, 1948. 403.

5 Goodman, L., and Gilman, A., *The Pharmacological Basis of Therapeutics*, The Macmillan Co., New York, 1941, 658.

6 Githens, T. S., *J. Pharm. and Exp. Therap.*, 1917, **10**, 327.

7 Youmans, J. B., and Trimble, W. H., *J. Pharm. and Exp. Therap.*, 1930, **39**, 201.

8 Rothlin, E., *Bull. de l'Acad. Suisse des Sci. Med.*, 1946-47, **2**, 249.

same pharmacologic effects as ergotoxine, also raised the body temperature when given in toxic doses by Rothlin. Small doses of ergotamine, on the other hand, resulted in a depression of body temperature. Rothlin's observations were made on the rabbit and also indicated that ergocristine and the other alkaloids of ergotoxine do not lower, but rather produce elevations of, body temperature.

Sawyer and Schlossberg[9] studied the effects upon the body temperatures of ergotamine-treated cats of exposure to warm and cold environments. They injected 0.5 mg per kg of ergotamine tartrate intramuscularly and found that their animals were then unable to regulate their temperatures as well in abnormal environments as were normal cats. The deleterious effect in the warm environment was regularly more pronounced than that observed in the cold.

Methods and Materials. Ergotoxine ethanesulfonate† has been used in our investigations of temperature regulation in albino rats. Ergotoxine was originally selected as a means of investigating this mechanism because of its having been reported to have a thermogenic effect when administered to certain animals and because its thermogenicity has been accounted for on the basis of central (hypothalamic) stimulation. It has, in fact, proven to be a satisfactory thermogenic agent in the white rat in spite of previous reports to the contrary (Githens[6]).

After considerable experimentation it was found that the most satisfactory solvent for the ergotoxine ethanesulfonate was dilute ethyl alcohol, prepared by diluting ¼ cc of 50% alcohol to 2 cc with distilled water. One mg of the drug was dissolved in this amount of solution. The dilute alcoholic solution, by itself, when injected intraperitoneally, failed to produce any effect upon body temperature. Although never used routinely, saline suspensions (1 mg ergotoxine in 2 cc physiological saline) and a solution of 1 mg of the drug in 2 cc of 1/1000 normal hydrochloric acid were found to be as effective in producing ergotoxine fever in our rats as the dilute alcoholic solution.

The dose of ergotoxine utilized in the experiments reported herein has been 4.5 mg per kg and this has been administered intraperitoneally. This dosage can be given without toxic effects although rats receiving it show considerable increase in respiratory rate and a rather general increase in skeletal muscular activity. Even when this dosage is combined with long periods of exposure to cold (5-8° C) the animals recover completely, and will survive several subsequent administrations of the drug, combined with cold stress.

As quickly as possible, after injection of ergotoxine, records of the body temperatures of the experimental animals and of suitable controls were started. The control animal, in each experiment, was simultaneously subjected to all the procedures, including cold stress, to which the experimental animal was subjected, except for the administration of ergotoxine. Temperatures were recorded from each of the animals at one-minute intervals by means of a 2-channel Brown electronic recording potentiometer and copper constantan thermopiles inserted into the colon to the level of the diaphragm as previously described.[10]

Four main types of experiments have been carried out to determine: (1) the effects of ergotoxine upon rats whose environmental temperatures remain within the normal range of 28 to 31° C; (2) the effects of transferring ergotoxine-treated animals to a cold environment (5-8° C) after their hyperthermias have run their course; (3) the effects of exposure to cold at the height of the ergotoxine fever; and (4) the effects of exposure to cold immediately after administration of ergotoxine.

The effects of subcutaneous injections of ergotoxine have also been observed and com-

[9] Sawyer, M. E. M., and Schlossberg, T., *Am. J. Physiol.*, 1933, **104**, 172.

† The ergotoxine ethanesulfonate was very kindly furnished by the Wellcome Research Laboratories, Tuckahoe, N. Y.

[10] Buchanan, A. R., and Hill, R. M., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 602.

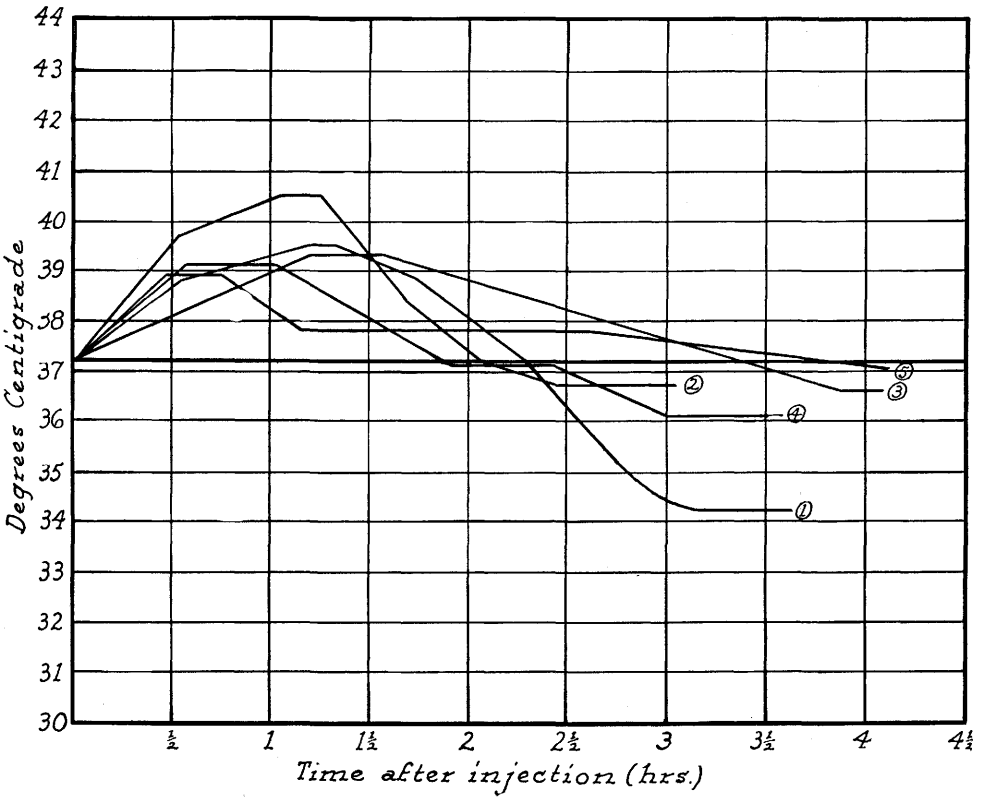

FIG. 1.
Graphic representation of the hyperthermic reactions recorded from 5 ergotoxine-treated rats in a 28-31° C environment.

pared with those produced by intraperitoneal administration. Ergotoxine (4.5 mg per kg) and urethane (0.25 g per kg) have been administered simultaneously in order to determine whether the typical ergotoxine fever is suppressed by general anesthesia. Mild heat stress has been applied to a considerable number of our ergotoxine-treated rats in order to learn whether the drug also interferes with the ability to regulate against heat.

Finally, the effects upon the internal temperatures of ergotoxine-treated rats of minimal changes in their external environmental temperatures have been investigated by utilizing a small incubator placed in the cold room. This arrangement has enabled us to produce at will any environmental temperature between that of the cold room (5-8° C) and that which is considered normal for our animals (28-31° C).

Wistar Strain rats from our own colony, as well as Denver University Strain rats, have been utilized in this study and the same results have been obtained in both. All have been regulating animals[10] and practically all have been fully developed adult rats.

Results. Intraperitoneal injections of ergotoxine ethanesulfonate in doses of 4.5 mg per kg have consistently resulted in significant rises in body temperatures of albino rats when the environmental temperature ranged between 28 and 31° C. The total hyperthermic reaction of a given animal, in degree-minutes, may be calculated from the total area under the graph in a continuous temperature record from the time of administration of the drug to the time when the temperature returned to its initial level. An environmental temperature of 31° was conducive to the development of more fever than one of 28°. If the experiment was continued in the same environment, beyond the period of ergotoxine hyperthermia,

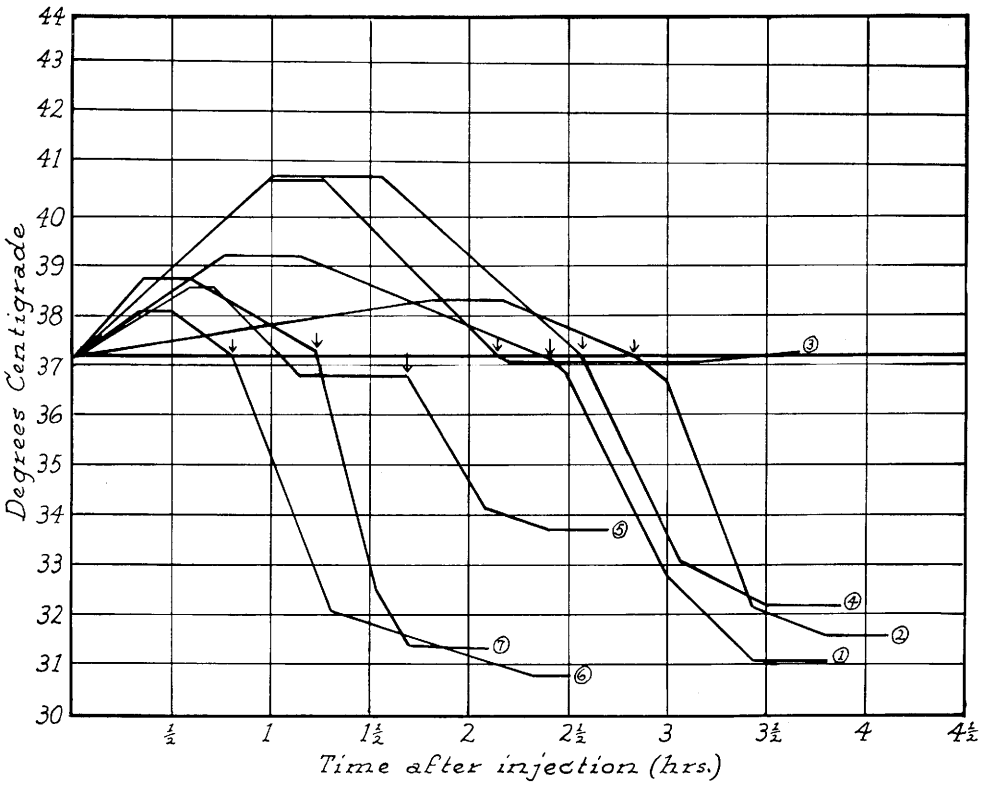

FIG. 2.

Graphic representation of hyperthermic reactions in 7 ergotoxine-treated rats and of hypothermia in the same animals as the result of exposure to the cold environment (5-8°C). The time at which each rat was exposed is indicated by an arrow.

the temperature of the rat fell below its initial (pre-hyperthermic) level.

The average hyperthermic reaction in 14 ergotoxine-treated rats, 12 of which are included in Fig. 1 and 2, amounted to 122 degree-minutes with a range from 26 to 283. The average maximum reading was 2° C above the initial temperature with a range from 1.1° to 3.6° and the average rate of rise was 3.1° C per hour (Fig. 1 and 2).

Placing ergotoxine-treated rats in the cold room (5-8° C) when their temperatures reached pre-hyperthermic levels resulted in rapid temperature falls (Fig. 2). These continued to rather low levels and their average rate was 9.2° per hour with a range from 6° to 15.6°.

Examination of Fig. 1, 2, 3, and 4 reveals that the time-temperature graphs obtained from our experimental rats consist essentially of linear segments with sharp inflection points. Curved segments have been indicated only where 10% of the points in the original record deviated 0.2° or more from a straight line. The temperature records have been simplified and their visualization made easier by shifting all of them as necessary to bring the initial temperature of all animals to a standard figure of 37.2° C.

Those rats transferred to the cold environment at the height of their fever (Fig. 3) have shown immediate falls in temperature which have proceeded at an average rate somewhat faster than that observed in animals placed in the cold environment at the termination of hyperthermia (10.2° C per hour).

The administration of ergotoxine in the same dosage to rats which are immediately exposed to the cold environment does not usually produce an increase in body tempera-

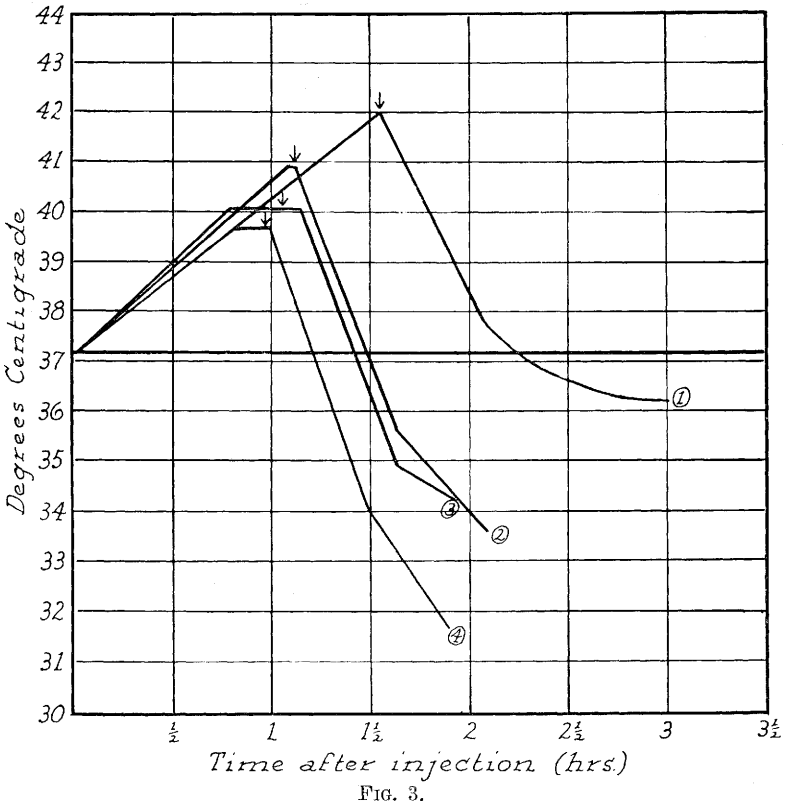

FIG. 3.

Temperature falls in 4 rats exposed in the cold room at the heights of their fevers. The times at which exposures began are indicated by arrows.

ture (Fig. 4). If the animals are placed in the cold environment (5-8° C) quickly enough after administration of the drug, there is no increase in temperature, but if there is a slight delay, a transient increase may appear; this is followed by a marked fall. For practical purposes, it may be stated that ergotoxine in the dosage which produces hyperthermia in an environmental temperature of 28-31° C results in a marked fall in body temperature when the animals are immediately exposed to cold. The average rate of fall in such animals is 7.1° C per hour, with a range from 3.9° to 9°.

Subcutaneous administration of ergotoxine to white rats in the same dosage and in the same medium described above, has not been sufficiently investigated for definite conclusions to be drawn. With the animal in a 28-31° C environment, it has so far always produced hyperthermia which is slower to appear, longer-lasting, and not as high as that produced by intraperitoneal injection.

Urethane (0.25 g per kg) has been given to a rat which had previously reacted to ergotoxine with a typical fever. The urethane, as such, had no effect upon body temperature. When, subsequently, the same animal received the same quantity of urethane together with the usual dose of ergotoxine, it failed to manifest any hyperthermia at an environmental temperature of 28-31° C; exposure to cold, however, resulted in a marked fall in temperature which was comparable to that seen in ergotoxine-treated animals without anesthesia. Subsequent injections of ergotoxine in other animals, and without urethane, have always produced hyperthermic reactions as marked as, or more marked than, that produced by the original injection.

Instability of regulation against mild heat stress has regularly been demonstrated in

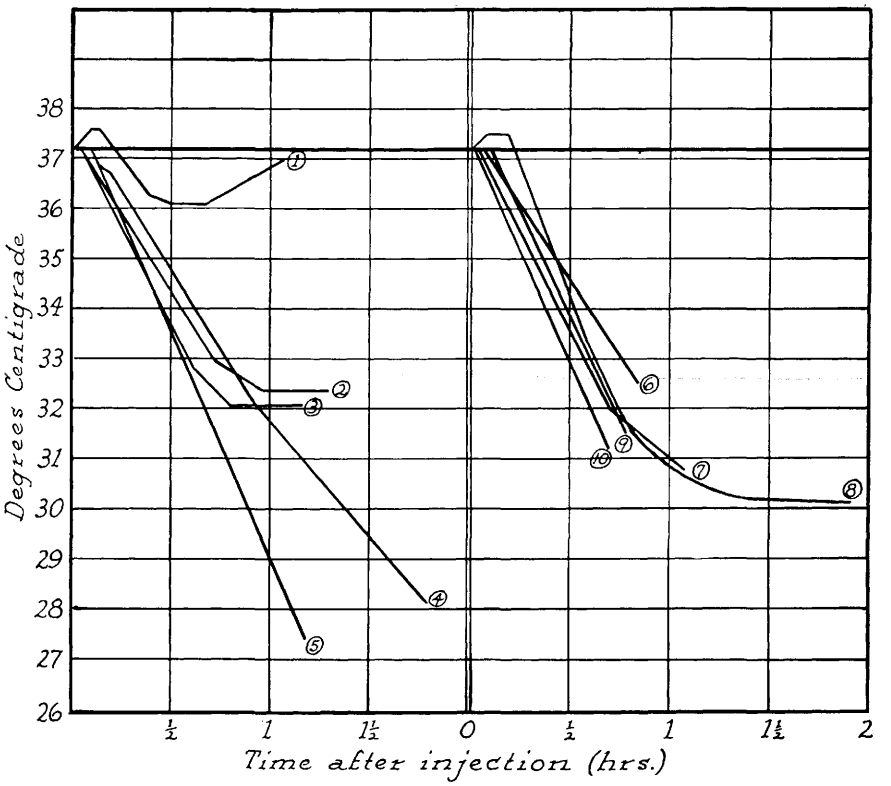

FIG. 4.

Temperature falls in 10 rats which were exposed to cold immediately after administration of ergotoxine. The animals have been separated into two groups only to prevent the confusion which would result from attempting to include all of them in a single graph.

animals given ergotoxine. It must be noted, however, that normal rats regulate poorly against heat and that the instability demonstrated in ergotoxine-treated animals is relative rather than absolute.

The marked sensitivity of experimental rats to changes in the temperature of their external environment has been particularly well demonstrated in those animals observed while in the incubator which, in turn, was operating in the cold room. It has been repeatedly shown, in such experiments, that the body temperatures of ergotoxine-treated rats placed in and maintained at incubator temperatures of 22-25° C may either rise or fall initially, after which they remain very constant within a rather narrow range. If the thermostat of the incubator is adjusted downward to such an extent that, after a considerable period of slow cooling, the new environmental temperature stabilizes at a level 3 degrees below its original level, the rat's internal temperature falls almost as rapidly as that of its environment and almost as much (2.5° C).

Discussion. Two possible explanations may be proposed as to why Githens[6] observed falls in the temperatures of white rats after administration of ergotoxine as contrasted to the rises which were consistently observed by us. The first of these relates to the difference that has been noted between rectal and high colonic temperatures. Hill *et all.*[11] have shown that this difference averages 3.5° C and that rectal temperatures are much more subject to modification by the external environment. They are probably also modified by the peripheral vasoconstriction said to result from the direct action of ergotoxine on

[11] Hill, R. M., Ware, A. G., and Schultz, F. H., *Cancer Research*, 1943, **3**, 839.

the smooth musculature of peripheral vessels. Githens' temperatures were rectal temperatures while ours were all obtained from the colon at or near the level of the diaphragm.

A second way in which the discrepancy between our results and those of Githens may be explained suggests itself in the light of our observations as to the extreme sensitivity of ergotoxine-treated rats to their environmental temperatures. Hyperthermia was a consistent result of ergotoxine administration to our rats in an environmental temperature of 28-31° C. When the drug was given to rats which were immediately placed in a 22-25° C environment, hyperthermia was absent or of slight degree and of short duration; in some animals there was an immediate fall. Githens may have conducted his experiments in a considerably cooler environment than that in which the majority of ours were conducted or may possibly have overlooked an early and transient rise in temperature, since his first reading was made 30 minutes after injection of ergotoxine.

Increases in temperature resulting from the administration of ergotoxine and ergometrine to albino rabbits were studied by de Beer and Tullar[12] who also analyzed the sources of error attributable to animal variation and environmental influences. With regard to the latter, they recognized that room temperature may alter the hyperthermal response, an observation which, as previously mentioned, was also made by Sawyer and Schlossberg.[9] The latter investigators, however, reported temperatures obtained with a clinical thermometer from the groin; such temperatures would certainly be affected by the peripheral vascular reactions ordinarily attributed to ergotoxine, by those due to the environmental temperature itself, and by a combination of both.

It is interesting to speculate as to whether the thermal changes observed by us and by previous investigators are due to direct action upon the hypothalamus and, if so, whether the effect is due to stimulation or depression of the heat-regulating centers therein. At the present time we are inclined toward the opinion that ergotoxine stimulates the hypothalamus directly and, so far as its heat-regulating centers are concerned, unselectively. Whether this stimulation is manifested by hyper- or hypothermia apparently depends to considerable degree upon the environmental temperature. It appears reasonable to assume, further, that our "normal" environment (28-31° C) provides just sufficient advantage for the heat-production and heat-conservation centers that the effects of their stimulation overbalance those of stimulation of the heat-loss center. A cool or cold environment, conversely, provides an advantage in favor of the heat-loss center and hypothermia results.

It is admitted that the above concept of the mechanisms responsible for the phenomena observed and reported herein, ignores the direct vasoconstrictor effect on peripheral vessels usually attributed to ergotoxine. One might, perhaps, assume that peripheral vasoconstriction occurs in the "normal" environment and thus contributes to hyperthermia while the "sympathetic reversal" of Dale,[13] due to increased secretion of adrenaline, may occur in cooler environments and result in peripheral vasodilatation; this reaction may be particularly pronounced when the animals are abruptly exposed to the cold environment (5-8° C).

Summary. Intraperitoneal administration of ergotoxine to albino rats has consistently resulted in hyperthermic reactions when the environmental temperature has been 28° C or higher. When ergotoxine-treated rats were placed in an environment whose temperature varied between 5° and 8° C, hypothermia always resulted; this was true of immediate exposure and of exposure subsequent to and during hyperthermia. Environmental temperatures between 22° and 25° C were sometimes conducive to moderate rises in temperature and sometimes to moderate falls; these changes were of short duration and were followed by long periods in which the body temperature remained within a very narrow range. Urethane given in conjunction with

[12] de Beer, E. J., and Tullar, P. E., *J. Pharm. and Exp. Therap.*, 1941, **71**, 256.

[13] Dale, H. H., *J. Physiol.*, 1913, **46**, 291.

ergotoxine eliminated the hyperthermic effect. Subcutaneous administration of ergotoxine gave rise to less dramatic hyperthermic responses and to hypothermia (in cold environments) that was essentially the same as that elicted by intraperitoneal injection.

The authors wish to thank Dr. R. W. Whitehead, Professor of Physiology and Pharmacology, University of Colorado Medical School, for valuable suggestions and criticisms.

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The Changes in the Electrocardiogram Associated with Standing.*

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Differences of opinions concerning the mechanism for the changes in the electrocardiogram that occur with standing are great. They are mainly due to the fact that some investigators were exclusively interested in the alterations which appeared immediately after assuming the erect position while others studied only those changes which occured after minutes of standing. There is agreement that the *immediate* changes are due both to the change of the position of the heart and to an altered contact between the heart and the neighboring structures which show different conductivity.[1,2,3] The alterations which appear later, *viz.*, during continued standing are attributed by some authors to an altered blood supply to the heart,[4] while others assume a change in the tonus of the sympathetic nervous system which modifies the repolarization processes in the heart. This latter contention is based chiefly on the finding that after administration of sympathicolytic drugs positional changes failed to appear in the electrocardiogram.[5,6]

We studied this problem on 80 male patients who had no evidence of organic heart disease. Electrocardiograms were taken with the patient in the supine position, immediately upon standing, after standing for 1 minute, 5 minutes, 15 minutes and again immediately upon lying supine. In 12 patients 0.5 mg of Dihydro-ergotamine 45 was given intravenously and records were again taken in the supine position at the height of the systemic effects and on standing.

Results. Of the 80 patients, 25 or 31.2% showed significant electrocardiographic changes on standing. Some of the changes occurred immediately upon standing while others took place many minutes after standing was maintained.

Fig. 1 and Fig. 2 show that the final deflection in the electrocardiogram may become higher or lower with prolonged standing. Changes may appear immediately, disappear later and reappear (Fig. 1) or they may be absent immediately on standing, but appear later (Fig. 2).

A further significant result of our studies was the temporary appearance of A-V rhythm with a change of position in 4 cases (Fig. 3) and the frequent appearance of a marked sinus arrhythmia.

Finally, the studies revealed that in 11 out of 12 cases the significant electrocardio-

* The expenses of this study were defrayed by a grant from Bernhard Altmann.

1 Sigler, L. H., *Am. Heart J.*, 1938, **15**, 146.

2 Scherf, D., and Weissberg, J., *Am. J. M. Sc.*, 1941, **201**, 693.

3 White, P. D., Chamberlain, F. L., and Graybiel, A., *Brit. Heart J.*, 1941, **3**, 233.

4 Akesson, S., *Upsala läkeref. förh.*, 1936, **41**, 383.

5 Nordenfelt, O., *Acta med. scand. Suppl.*, 1941, **119**, 1.

6 Wendkos, M. H., *Am. Heart J.*, 1944, **28**, 549.