nificantly increased and testes weight significantly reduced over that of room temperature controls. In general, however, no significant difference in body weight or gross appearance was observed between the various groups either under cold room or room temperature conditions.

Discussion. Available data indicate that requirements for a number of nutrients are markedly increased under conditions of low environmental temperature. This is particularly true for some of the water-soluble vitamins. An increased requirement for thiamine(5,6), pyridoxine(7), and ascorbic acid (8) has been demonstrated following prolonged exposure to low environmental temperatures. Requirements are also increased for vit. A(9). Previous findings indicate that the survival time of vit. A-deficient rats is markedly reduced under conditions of low environmental temperature(9). Results of the present experiment indicate that a transient pre-test deficiency of vit. A (apparently corrected by the administration of relatively high doses of vit. A) may also impair the ability of rats to withstand subsequent exposure to cold. Available data, however, do not indicate what factors are responsible for the lowered resistance.

Findings of the present experiment suggest that transient nutritional deficiencies may leave residual effects in the organism which normally are not readily apparent but which may become manifest under conditions of stress. Further experiments are indicated to determine the residual effects of transient nutritional deficiencies (of other nutrients as well as vitamin A) and the aggravating effect of stress factors thereon.

Summary. A transient deficiency of vit. A, apparently corrected by the administration of relatively high doses of this vitamin, markedly impaired the ability of rats to withstand subsequent exposure to cold.

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Effects of Atropine on Brain Respiration.* (18280)

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The pronounced central nervous disturbances of atropine poisoning and the use of the drug in the treatment of postencephalitic Parkinsonism suggest the desirability of an examination of its metabolic action on central nervous tissues. It has been shown *in vivo*(1) that atropine depresses the oxygen consumption of brain. *In vitro* experiments on the effects of atropine on other tissues, *e.g.* muscle(2), have produced negative results. However, data on the effects of the alkaloid on the *in vitro* respiration of brain homogenates are lacking. This paper describes some effects of atropine on the respiration of rat brain homogenates.

Methods. Rats of mixed strains were used. After decapitation the brains were removed, weighed and homogenized in glass homogenizers(3). The homogenates were made directly in the complete medium used, the only

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TABLE I. Effect of Atropine on Endogenous Respiration in Dilute Buffered Saline.

pH	Drug	Q_{O_2}	% inhibition
6.0	—	4.8	—
	ACh*	4.64	3.3
	At†	4.66	2.9
	AA‡	4.33	6.6
	—	2.99	—
	At	2.89	3.3
7.0	—	3.03	—
	At	2.69	11.1
	—	4.85	—
	At	4.30	11.3
8.0	—	5.43	—
	ACh	5.39	0.7
	At	3.77	30.6
	AA	3.69	32.1
	—	3.02	—
	At	1.60	47.0

Medium. 0.033 M phosphate buffer; NaCl to give ionic strength 0.2. Added drugs: * acetylcholine-0.01 M; † atropine sulfate-0.001 M; ‡ acetylcholine chloride-0.01 M together with atropine sulfate-0.001 M. All concentrations refer to final concentrations.

further addition being the desired amount of alkaloid. The media were dilute buffered saline (Table I), Krebs-Ringer phosphate(4) and Robinson's medium(5). Respiration of the homogenates was measured in the standard Warburg apparatus at 37.7°C. The gas phase in all cases was air. Readings were commenced after a 5-minute equilibration period and were made at 10-minute intervals over periods of 90 to 120 minutes. The spectrophotometric data were obtained using the Beckman quartz spectrophotometer.

Results. Fig. 1 and 2 show clearly the inhibitory action of atropine and the partial reversal of the inhibition by acetylcholine. It should be noted that the data from which these curves were constructed were obtained under conditions of complete media with respect to inorganic ions and with glucose as substrate.

The effects of pH and acetylcholine addition (Table I) were particularly interesting in the light of experiments on invertase inhibition by atropine(6). In the case of invertase a similar progressive increase in inhibitory action with increasing pH was observed.

A study of the absorption spectrum of atropine under the same conditions of pH and ionic strength as prevailed in the respiration experiments mentioned above showed successive increases in optical density with pH in the position of the first maximum at 245-49 mμ (Fig. 3). It will be noted that the increment for the step in pH from 7 to 8 was greater than that for the preceding pH increase from 6 to 7. Similarly, there was a greater increase in inhibition of brain respiration corresponding to the first mentioned pH increase than for the latter. The significance of this is not clear.

The effects of acetylcholine in the case of the dilute buffered saline in the absence of substrate were either absent or slightly depressant. Although no systematic study of ionic effects has been

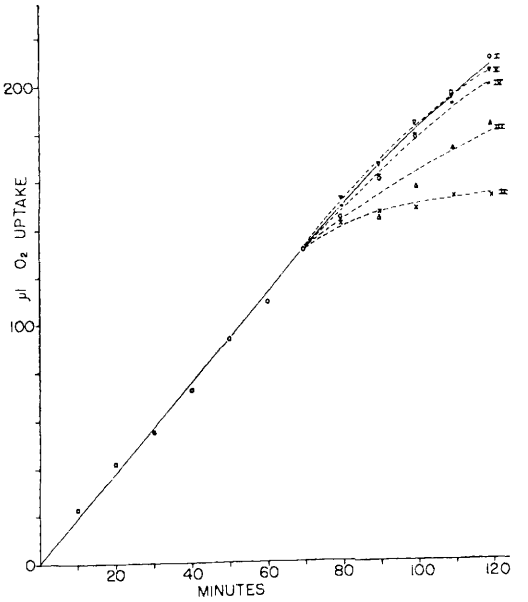

FIG. 1

The inhibition of respiration by atropine and partial reversal of the inhibition by acetylcholine. System: 10% homogenates of whole brain in Robinson's medium (1949). Additions of atropine sulfate or acetylcholine chloride made at 70 min. I. Normals; II. 10^{-2} M atropine added; III. 10^{-3} M atropine added; IV. 10^{-2} M atropine and 10^{-2} M acetylcholine added together; V-10^{-3} M atropine and 10^{-2} M acetylcholine added together. 37.7°C, pH 7.4. All concentrations refer to final concentrations.

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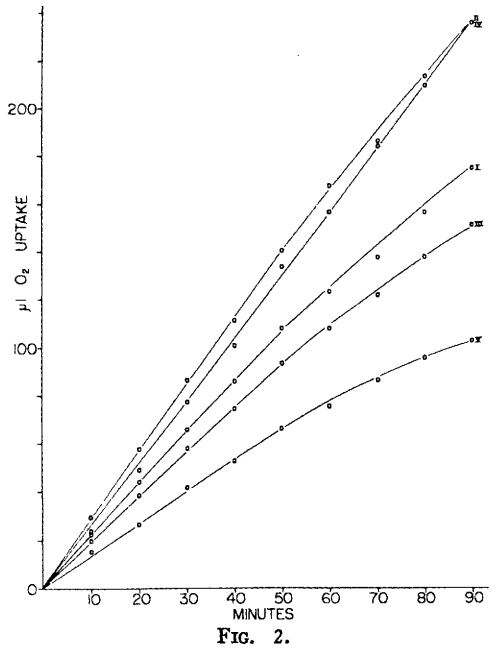

FIG. 2.

Effects of atropine and acetylcholine on eserinized homogenates. System: 10% homogenates of whole brain in Robinson's medium (containing glucose) incubated with acetylcholine chloride, atropine sulfate, and physostigmine salicylate before readings were made. I. No addition of drugs; II. 10^{-4} M physostigmine added; III. 10^{-2} M atropine, 10^{-2} M acetylcholine, and 10^{-4} M physostigmine added together; IV. 10^{-2} M acetylcholine and 10^{-4} M physostigmine added together; V. 10^{-2} M atropine only added. 37.7°C, pH 7.4. All concentrations refer to final concentrations.

carried out, some additional data appear in Table II which, considered with the previously presented data, seemed to indicate that the potassium ion was important in the acetylcholine reversal effect. Apparently an added substrate was also necessary for acetylcholine reversal. Incomplete as these results are they suggest that there is a considerable degree of complexity in the factors influencing the inhibition of respiration by atropine.

The stimulatory effect of physostigmine (Fig. 2) was also noted in washed homogenates with pyruvate as a substrate (unpublished).

Since added acetylcholine in the absence of a cholinesterase inhibitor failed to affect oxygen consumption, it seemed unlikely that the oxidation of products of acetylcholine hydrolysis was responsible for the reversal effect.

Discussion. In addition to the rather weak inhibitory action of atropine on brain respiration a point of particular interest was the observation of an atropine-acetylcholine antagonism in a metabolic sense and the possible significance of this antagonism in a system lacking structural integrity. It is difficult to explain the actions of these drugs in this case on the basis of competition for 'receptors' when the term is used with any implied or explicit morphological meaning(7). These results are of interest regarding Welsh's(8) suggestion that acetylcholine may function as a co-factor in some enzymatic process.

The pH dependence of atropine inhibition is clearly indicative of the unionized amine being the inhibitory form of the molecule. Much additional information is required to

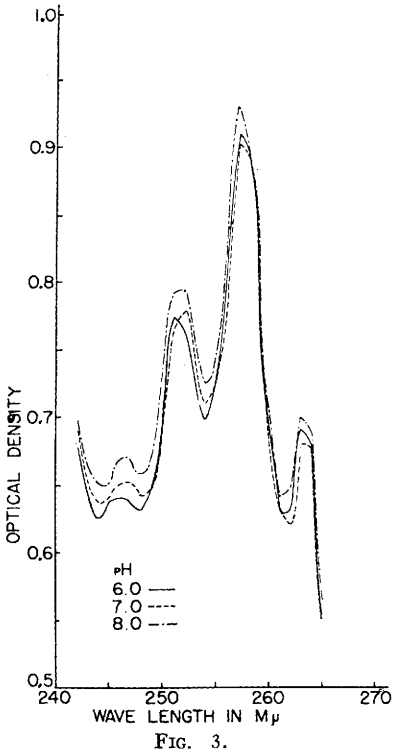

FIG. 3.

The absorption spectrum of atropine sulfate as a function of pH. Solutions of atropine were made in 0.033M phosphate buffers containing NaCl to make the ionic strength 0.2. Blanks were the corresponding saline buffers without atropine. The concentration of atropine was 10^{-2} M.

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TABLE II. Effect of Atropine on respiration supported by Alpha-glycerophosphate in Various Media.

Medium	Drug	% inhibition
I	10^{-3} M At*	41
II	10^{-2} M At	23
	10^{-2} M AA†	7
III	10^{-3} M At	15
	10^{-2} M At	41

Medium I. Ca-free Krebs-Ringer Phosphate with NaCl replaced by KCl; medium II. Krebs-Ringer Phosphate, Ca-free; medium III. Robinson's medium (see ref. 4) (similar to Krebs-Ringer but with somewhat higher buffer concentration). *Atropine sulfate; †Equimolar atropine sulfate and acetylcholine chloride together. Alpha-glycerophosphate was 0.01 M in all cases. All concentrations refer to final concentrations in the test system.

relate the spectroscopic changes to the ionization and corresponding respiration inhibition if such a relationship exists between all 3 factors. An obvious difficulty is the requisite demonstration of the manner in which ionization of the amine moiety affects the tropic acid group which is chiefly responsible for the absorption characteristics of the whole molecule(9). The importance of the ionic state of inhibitors has been emphasized in studies on cyanide and azide(10,11,12) and on certain antiseptics(13).

Summary. (1) It was found that atropine in relatively high concentrations inhibited the respiration of rat brain homogenates by as much as 40% under optimal conditions. (2) The respiration inhibition was partially reversible by acetylcholine under certain conditions. (3) The inhibition was pH dependent, increasing with increasing pH. Corresponding changes in the atropine absorption spectrum with varying pH were observed.

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The Growth-Promoting Action of Various Supplements in the Hyperthyroid Rat.* (18281)

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Studies on the deficiencies of rations composed principally of corn and soy-bean-oil meal for normal reproduction and lactation in rats and swine have been in progress at this station for several years. In the course of these investigations, it was deemed desirable to undertake the development of an assay procedure that was less time-consuming and more sensitive than that involving reproduction. One of the procedures used by other workers has been assessment of the ability of a supplement to reverse the growth inhibition induced by feeding thyroid-active agents to the immature rat. In the presence of an ample supply of other nutrients, it has recently been shown by Emerson(1), by Betheil and Lardy(2),

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