mide, biotin and riboflavin to prevent the achondroplastic effect of thallium, would suggest that thallium has a separate mechanism of action, although it is possible, on a more complete analysis, that some of these agents may act ultimately on the same site in affecting bone development.

Along with the genetic and nutritional deficiency technics for producing achondroplasia, thallium is now demonstrated to act in a highly consistent manner to produce a severe and uniform defect in the osseous development of the chick embryo.

Summary. Thallium sulfate produces a consistent and severe degree of achondroplasia in the chick embryo when injected on the fourth day of incubation. This abnormality appears to be similar to the genetic, and to some of the nutritional deficiency, types of chondrodystrophy. Thallium induced achondroplasia is not prevented by treating the embryo with manganese, biotin, nicotinamide or riboflavin, deficiencies of which have been reported to result in some type of chondrodystrophy in the chick embryo.

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Anesthetic Effects in Mice and Dogs of Some Unsaturated Hydrocarbons and Carbon-Oxygen Ring Compounds. (17647)

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Previous reports of anesthesia with butylene stated that the butylene used was a mixture of isomers(1) or that it was probably chiefly ethyl ethylene(2). Effects observed using butylene could not therefore be attributed to any one isomer. The recent development of efficient distillation procedures has made possible the separation of various hydrocarbons with a purity hitherto unattainable. This improvement has permitted a more definitive attempt to find relationships between chemical constitution and physiological activity.

Methods. The methods used have been described(3). Essentially the experiments were carried out in a large jar equipped through the rubber stopper with apparatus that provided for introduction of known quantities of oxygen or anesthetic compound while maintaining atmospheric pressure. Carbon dioxide was absorbed by soda lime. The oxygen concentration was kept at 21%. The experiments were carried out between 25 and 27° C.

Properties of the compounds used are given in Table I.

In order to standardize results the experiments were limited to concentrations of anesthetic agents that would afford surgical anesthesia within 10 minutes. Experiments were terminated after 20 minutes of anesthesia. Surgical anesthesia was accepted as the condition in which the righting reflex had been lost and in which the animals failed to respond to pinching a leg against the side of the jar with a stirring rod. At least 64 mice were used to determine each percentage value given in Table I. Probit analysis of results(4) was used in order to minimize the number of animals necessary from which to obtain statistically reliable values, for several of the materials tested were available in small quantities. To test the degree of sensitization of

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2. Brown, W. E., *J. Pharm. and Exp. Therap.*, 1926, v27, 1.

3. Virtue, R. W., *Anesthesiology*, 1949, v10, 318.

4. Finney, D. J., *Probit Analysis, A Statistical Treatment of the Sigmoid Response Curve*, Cambridge University Press, 1947; Bliss, C. I., and Cattell, McK., *Ann. Rev. Physiol.*, 1943, v5, 479.

TABLE I.
Anesthetic Effect in Mice and Dogs of Some Unsaturated Hydrocarbons and Carbon-Oxygen Ring Compounds.

Compound	Purity, %	Boiling point, °C	% in atmosphere for: Surgical anesthesia	% in atmosphere for: Respiratory arrest	Anesthetic index
Butene-2, cis, H.B.	96.18*	4	17.2	25.5	1.5
Butene-2, trans, L.B.	98.92*	1	18.7	21.0	1.1
Butene-1	99.88†	—6	22.7	27.2	1.2
Isobutylene	99.44*	—7	19.8	32.0	1.6
Butadiene-1,3	99.87*	—4	17.3	25.7	1.5
Pentene-1	99.34*	30	8.6	9.7	1.1
Propyne	97 plus	—105	Indeterminate	18.7	—
Butyne-1	97 plus	18	10.8	19.5	1.8
Butyne-2	97 plus	29	4.1	4.6	1.1
Pentyne-1	97 plus	40	6.9	14.1	2.0
Pentyne-2	97 plus	56	5.2	9.3	1.8
Heptadiyne-1,6	97 plus	108	2.0	4.1	2.05
Cyclopentene	99.76*	45	5.0	7.7	1.5
Cyclohexene	98	83-85	2.9	6.0	2.1
Trimethylene oxide		46.7-47.7	13.4	20.0	1.5
Pentamethylene oxide (Tetrahydropyran)	99	88	4.67	4.74	1.02
Dihydropyran	95	86	2.6	4.3	1.7
Dioxolane-1,3		74	14.3	19.3	1.3
2-Methyl dioxolane-1,3		81	11.4	17.1	1.6
4-Methyl dioxolane-1,3		85	12.8	14.6	1.1

* National Bureau of Standards, freezing point method.
† National Bureau of Standards, mass spectrometer method.

cardiac tissue to epinephrine by anesthetic agents, dogs were anesthetized and epinephrine was injected according to the technique of Meek, *et al.*(5).

Results. Induction of anesthesia with members of the ethylene series was accompanied by tachypnea and mild tremors of legs and head. A few tremors of hind legs were seen during anesthesia. Sleep was generally quiet but the mice were not well relaxed. Recovery was characterized by wild excitement lasting from 15 to 30 seconds. Induction with members of the acetylene series produced a greater state of agitation than in the ethylene series. Stimulation during induction with propyne was followed by convulsive movements. Continuous tremors and tonus of the tail appeared and were so extensive that one could not tell when surgical anesthesia was obtained. Surgical anesthesia with other acetylenic substances was not quiet, one or more legs moving constantly. Cyclopentene and cyclohexene produced only mild excitement during induction, and surgical anesthesia was quiet with the mice well relaxed. When respiratory arrest occurred it was preceded by convulsive movements. Induction was slow with all the carbon-oxygen ring compounds except di- and tetrahydropyran (pentamethylene oxide). With all this series induction was characterized by depression without stimulation of the nervous system.

Four mice anesthetized for 10 minutes with trimethylene oxide required 68 minutes for recovery. On the day following anesthesia all the animals that had been exposed were found dead. Sections were made of their brain, heart, lung, liver and kidney tissues. Congestion and acute hyperemia of the lungs were demonstrated microscopically.

With the exception of the mice inhaling trimethylene oxide, all the animals that had been anesthetized with the 20 compounds tested appeared healthy thereafter for a period of at least 2 weeks. With all the compounds tested, respiratory arrest preceded circulatory failure.

Electrocardiograms were made during injection of epinephrine when dogs were anesthetized with each of the following: butene-1;

5. Meek, W. J., Hathaway, H. R., and Orth, O. S., *J. Pharm. and Exp. Therap.*, 1937, v61, 240.

cis butene-2; isobutylene; pentene-1; butadiene-1,3; cyclopentene; cyclohexene; propyne; and butyne-1. Arrhythmias were produced with each agent. The electrocardiograms using propyne were so abnormal before injection of epinephrine that it was difficult to say whether epinephrine caused a change. The arrhythmias were least significant with isobutylene, for in 4 dogs this substance produced only mild tachycardia and minor voltage changes. Results similar to those found here have been reported for butyne-1, cyclopentene, and cis trans butene-2 by Carr and Krantz(6).

Discussion. Three compounds gave anesthetic indices of 2 or higher: pentyne-1, cyclohexene, and heptadiyne-1,6. The disagreeable odor and the central nervous signs including tremors and convulsions eliminated pentyne-1 from clinical consideration. Convulsive movements were observed with cyclohexene just prior to respiratory arrest, which in addition to its odor and low volatility also eliminated its clinical consideration. Heptadiyne-1,6 had a strong odor of acetylene and was difficult to volatilize.

The concept that narcotic potency increases with molecular weight(7) was generally supported. However, butyne-2 required 4.1% while pentyne-2 took 5.2% concentration for surgical anesthesia. Other factors than concentration may have been responsible for this discrepancy, for both substances were powerful nervous system stimulants. The results also support the concept that anesthetic potency increases with degree of unsaturation(7). The outstanding observation of these comparative studies was that the position of unsaturation in the molecule appeared to have a substantial effect on the concentration required for both anesthesia and respiratory arrest, and on the degree of central nervous stimulation. Compounds with unsaturation in the 2 position were definitely more potent than the corresponding compounds with unsaturation at the end of the chain. This was true with both the ethylene and acetylene series of compounds.

The carbon-oxygen ring compounds were toxic rather than being anesthetic agents. Henderson(8) noted congestion and mottling of lungs after his mice had been exposed to tetrahydrofuran. Stehle(9) found universal hyperemia of parenchymatous organs after giving ethylene oxide. Trimethylene oxide produced congestion and mottling in the lungs of mice examined here. Mice that had been exposed to tetrahydropyran did not show these changes.

Summary. 1. Anesthesia was induced in mice with 20 different compounds which were highly purified unsaturated hydrocarbons and carbon-oxygen ring compounds. Concentrations required for surgical anesthesia and for respiratory arrest were measured.

2. Compounds with unsaturation in the 2 position in either the ethylene or acetylene series exhibited greater physiological activity than did corresponding compounds having unsaturation in the terminal position.

3. Trimethylene oxide and 5 and 6 membered carbon-oxygen ring compounds were toxic.

4. Where sufficient agent was available, sensitization of cardiac tissue of anesthetized dogs to epinephrine was determined. Irregularities of cardiac rhythm of at least moderate severity were produced with all 9 agents used except isobutylene which caused only mild tachycardia and minor voltage changes.

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6. Carr, C. J., and Krantz, J. C., Jr., *Fed. Proc.*, 1949, v8, 279.

7. Adriani, J., *The Pharmacology of Anesthetic Drugs*, C. Thomas, Springfield, 1941.

8. Henderson, V. E., and Smith, A. H. R., *J. Pharm. and Exp. Therap.*, 1936, v57, 394.

9. Stehle, R. L., Bourne, W., and Lozinsky, E., *Arch. f. exp. Path., u. Pharm.*, 1924, v104, 82.

York City, who supplied the dioxolanes; the E. I. du Pont de Nemours Company of Wilmington, Del., for the dihydropyran and tetrahydropyran; and to Dr. Robert Stickler of the Department of Surgery, State University of Iowa, who courteously prepared and examined microscopic sections of the tissues.

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Anatomic Changes Produced in Mice Treated with Excessive Doses of Cortisone. (17648)

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Hench, Kendall, Slocumb, Polley, Barnes and Smith(1,2) reported remarkable effects of Cortisone in patients with rheumatoid arthritis and rheumatic fever. These findings suggested the study of effects of large doses of Cortisone in animals. Accordingly, a dose equivalent to 50 times the amount used for humans was administered subcutaneously to mice. The limited quantity of Cortisone available sharply restricted our dose range and the number of animals treated. As additional amounts of Cortisone become available, we plan to use varying doses and different species.

Method. Two groups of mice were used. A) Nineteen (male and female) immature mice (21 to 30 days old) received daily subcutaneous injections of 1.25 mg Cortisone, suspended in 0.1 cc saline, for 6 successive days, unless they died or were sacrificed earlier as indicated below. Twenty comparable controls received 0.1 cc saline only on the same days by the same route. B) Seventeen mature (male and female) mice weighing 19-26 g received daily subcutaneous injections of 2.5 mg Cortisone suspended in 0.1 cc saline. They were thus injected for 9 days, unless they died or were sacrificed earlier as indicated below. Twenty comparable controls received 0.1 cc saline only for the same time by the same route. The mice were kept in an air conditioned room the temperature of which ranged from 79-85°F. They received water and Rockland Farm pellets *ad lib.*

Results. Of the 19 immature (21 to 30 day old) mice, one treated mouse with its corresponding control was sacrificed on the 2nd, 3rd, 4th, 7th, 9th, 10th, and 11th day after initiation of treatment. One died on the 5th day, one on the 6th day, five on the 8th day, 2 on the 9th day and one each on the 11th, 12th and 15th day. Of the 17 mature mice, one treated mouse and its corresponding control, was sacrificed on the 3rd, 9th and 17th day, after initiation of treatment, and one mouse died on the 5th, 7th, 9th, 16th, 17th and 19th day. Three died on the 8th, day, 3 on the 11th day, and 2 on the 13th day.

Some of the treated animals gained weight for one to 2 days and then lost weight; others lost weight from the outset. Those animals which died showed a precipitous weight loss during their last days. The average weight change of the immature group is shown in Fig. 1. This shows the loss of weight in the treated animals in contrast to the controls which gained weight. The results were essentially alike in the mature mice. Loss of body weight following Cortisone administration was reported by Kendall(3) and Kocha-

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3. Kendall, E. C., *Conference on Metabolic Aspects of Convalescence Including Bone and Wound Healing,* 10th Meeting, 1945, p. 87.