in the tissue culture cells are of the order sometimes reached in treating patients but those required for complete suppression of growth are well beyond clinical experience.

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Production of Achondroplasia in the Chick Embryo with Thallium.* (17646)

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(Introduced by C. P. Rhoads.)

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The "4-amino" derivatives of folic acid have occasionally produced alopecia in man(1). Since thallium is also a systemically-acting depilatory(2,3), is was of interest to determine whether these two agents had other similar biological effects. The "4-amino" derivatives of folic acid produced a marked inhibition of growth of the chick embryo(4). It was, therefore, decided to study the effects of thallium on the same system.

Methods. Fertile White Leghorn eggs were obtained from a commercial source. The eggs were incubated at 38°C and 85% relative humidity. Thallium sulfate was made up in distilled water as a solution containing 10 mg/cc. This solution (the usual volume was 0.05 to 0.20 cc) was injected into the yolk sac of the 4 day embryo, except for a few experiments using 2, 10 and 12 day embryos. The eggs were candled daily, and most of the embryos were sacrificed at 18 to 21 days of age. Some of the embryos were cleared and their skeletons stained with alizarin red S by the method described by Couch, *et al.*(5). The protective value of manganese sulfate, riboflavin, biotin and nicotinamide against thallium was examined by injecting each of these agents separately, but simultaneously, with thallium sulfate into the yolk sac of the 4 day embryo.

Results. The acute (0-4 days after injection) LD_{50} of thallium sulfate for the 4 day embryo is about 1.3 mg/egg. Most of the embryos surviving the acute lethal effect of 1 mg/egg lived to 18 to 22 days of incubation, although none hatched (Table I). Thallium produced a severe and consistent picture of achondroplasia in the chick embryo, which was evident in embryos sacrificed or dying at 10 days of incubation or later. At the larger doses of thallium (1 to 2 mg/egg), achondroplasia was associated with a moderate to severe degree of growth inhibition. At the smaller dose of 0.5 mg/egg, growth inhibition was negligible, although a severe achondroplasia was frequently present. The gross appearance of the affected embryos is shown in Fig. 1. The embryos injected at 4 days of incubation showed a marked degree of achondroplasia, whereas those treated after 10 to 12 days of incubation showed a less severe but definite inhibition in the growth of the extremities. The severely achondroplastic embryos (treated at 4 days) showed very short limbs with feet that were of normal size

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1. Burchenal, J. H., Karnofsky, D. A., Southam, C. M., Myers, W. P. L., Craver, L. F., Dargeon, H. W., and Rhoads, C. P., to be published.

2. Heyroth, F. F., *Thallium, a Review and Summary of Medical Literature,* Public Health Reports, Supplement No. 197, 1947.

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

4. Karnofsky, D. A., Patterson, P. A., and Ridgway, L. P., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 447.

5. Couch, J. R., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G., *Anat. Rec.,* 1948, v100, 29.

TABLE I.
Toxicity of Thallium Sulfate to 4-Day-Old Chick Embryo, and Incidence of Achondroplasia in Surviving Chick Embryos.

Dose, mg/egg	No. embryos	Acute toxicity (0-4 days after injection)	Mortality of embryos (death, or sacrifice ( ). Age of embryos, days)												Incidence achondroplasia, %
			9	10	11	12	13	14	15	16	17	18	19	20	21	
2.0	16	14					(2)									100
1.0	64	15			1	3(5)	(4)	1		(3)		3(6)	1	1	6(15)	92
0.5	15	4										(3)			6(2)	45*
0.2	5	1		2	(1)							(1)				0

* These embryos were within normal weight limits, and the achondroplasia was less severe than that occurring at 1 mg/egg.

or larger, principally due to edema, since the bones were small, parrot beak, thick trunk and a small head. On gross inspection of the head, the eyes appeared disproportionately small and the brain was small and seemed less solid than normal, particularly the cerebellum, which was atrophied. The abnormalities in eye and brain development were presumably due to compression of these organs due to the inhibition in skull growth. The feathers appeared to be well developed.

The normal and achondroplastic specimens, shown in Fig. 2, clearly show the defect in bone growth. The bones all appear to be present, but they are much shorter than normal, and the tibiae and femora are strikingly curved. Thallium sulfate did not produce achondroplasia when given at the maximum tolerated dose to the 2 day old embryo. However, the LD_{50} was estimated to be about 0.3 mg/egg, which is less than the amount of thallium sulfate necessary to consistently produce achondroplasia at 4 days.

The acute LD_{50} of thallium sulfate in the 10 to 12 day embryos (0 to 4 days after injection) was 2 mg/egg. The embryos surviving to hatching showed shortening of their extremities (Fig. 1) but the long bones of the legs were not curved, as seen on x-ray examination. Achondroplasia was produced by thallium nitrate at doses similar to those of thallium sulfate; in fact, the former preparation seemed to be more selective than thallium sulfate in its achondroplastic as compared to its lethal effect on the embryo. Ferrous sulfate and zinc sulfate were not toxic when injected into the yolk sac of the 4 day embryo at 5 mg/egg. These data indicate that the sulfate ion is not responsible or essential for the occurrence of achondroplasia.

Because of the reports that manganese(6,7), biotin(5,8) and riboflavin(9) deficiencies in the egg, and the injection of a nicotinic acid

6. Lyons, M., and Insko, W. M., Jr., *Kentucky Agr. Exp. Sta. Bull.*, 1937, No. 371, 61.

7. Caskey, C. D., and Norris, L. C., PROC. SOC. EXP. BIOL. AND MED., 1940, v44, 332.

8. Cravens, W. W., McGibbon, W. H., and Sebesta, E. E., *Anat. Rec.*, 1944, v90, 55.

9. Romanoff, A. L., and Bauernfeind, J. C., *Anat. Rec.*, 1942, v82, 11.

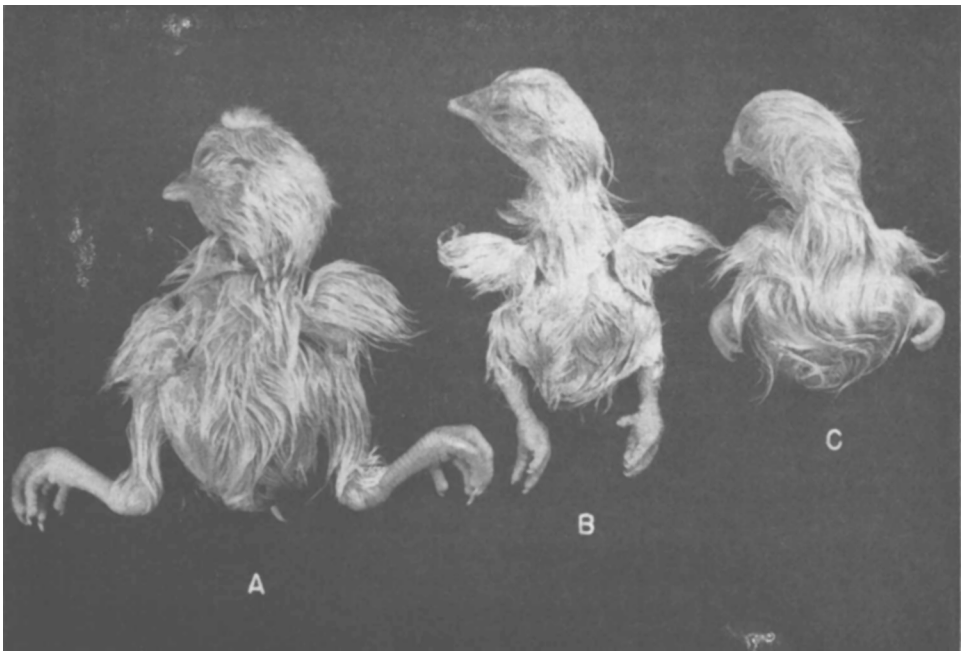

FIG. 1.

Gross appearance of chick embryos sacrificed at 21 days, immediately before hatching. A. Normal embryo. B. Embryo injected with 1.8 mg thallium sulfate at 12 days incubation. The skull and beak are not remarkable, but the extremities are considerably shortened. C. Embryo injected with 1 mg thallium sulfate at 4 days. This shows the characteristic picture of parrot beak, small head and eyes, short trunk, short extremities and feet of approximately normal size. There is over-all stunting of this embryo.

antagonist, 3-acetylpyridine(10) produce some form of achondroplasia in the chick embryo, the protective values of these agents in the chick embryo were tested against thallium. Four day embryos, treated with 1 mg/egg of thallium sulfate, were injected at the same time with either 5 mg manganese sulfate, 5 mg nicotinamide, 2 and 5 mg of biotin or 1 mg of water-soluble riboflavin (Lederle). The addition of thallium and one of these agents near their maximum tolerated doses caused a large percentage of early deaths in the embryos, but 3 to 5 embryos survived to 18 to 22 days in each group, and these all showed the characteristic and unmodified picture of "thallium" achondroplasia.

Discussion. Thallium has been found to exert a highly specific effect on the development of the chick embryo, by producing a severe degree of generalized achondroplasia. This effect does not occur if thallium is injected at the 2nd day of incubation, presumably because thallium is lethal to the 2 day embryo at a dose below that necessary to produce achondroplasia. At 4 days and later, the injection of thallium will interfere with bone development, the severity of the effect being related to the stage of embryonic development at the time of treatment. The localization of thallium in the chick embryo, the histology of the abnormal skeleton and changes in the embryo secondary to the failure of skeletal development will be the subjects of further studies. The achondroplasia produced by thallium appears to be similar to that described in hereditary chondrodystrophy in the chick embryo, and this has recently been analyzed as due to an autosomal recessive gene by Lamoreux(11). In the severe cases, parrot beak and curved tibiae

10. Ackermann, W. W., and Taylor, A., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 449.

11. Lamoreux, W. F., *J. Heredity*, 1942, v33, 275.

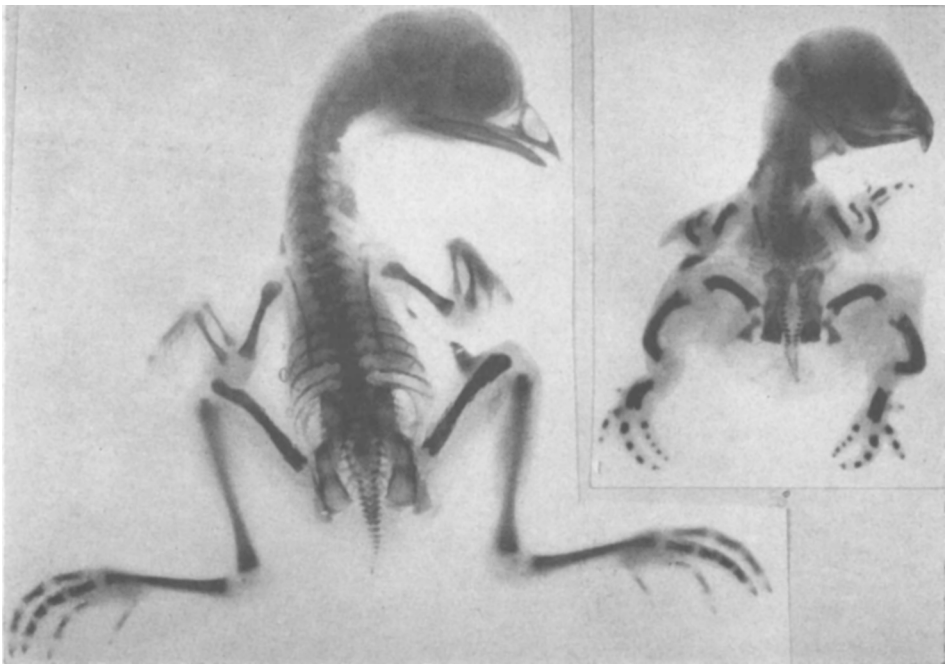

FIG. 2.
Skeletal structure of control and thallium-treated (1 mg/egg injected at 4 days) 21-day-old chick embryos. The diminution of skeletal growth is marked, and the curved femur and tibia are evident.

were present, and the embryos rarely hatched. While some of the embryos treated with thallium resembled the photographs published by Lamoreux(11), thallium can produce a more severe degree of achondroplasia. It is of great interest that a chemical can simulate a genetic abnormality, believed to be due to a single gene. Couch, *et al.*(5) and Cravens, *et al.*(8) have observed a high incidence of chondrodystrophy in the embryos from hens placed on a biotin-deficient diet, and this defect was prevented by injecting the egg with biotin. These embryos, as shown in published photographs(5,8), are also similar to the thallium-treated embryos. The sporadic occurrence of chondrodystrophy similar in type to the above—and the first reported instance of chondrodystrophy in the chick embryo—was described by Landauer and Dunn(12).

Micromelia and beak deformities have also been produced by the injection of insulin into the yolk sac of the 4 and 5 day old chick embryo(13), by sulfanilamide(14), by the nicotinic acid antagonist 3-acetylpyridine(10) and by the production of a riboflavin deficiency in the egg(9). Landauer(15) and Zwilling(16) have suggested that the micromelic action of insulin may be related to its hypoglycemic activity in the embryo, and this effect can be prevented by the administration of nicotinamide at the same time as the insulin. The micromelic effect of 3-acetylpyridine may also be related to this mechanism, and it is also of interest that the micromelia produced by sulfanilamide, although not related to hypoglycemia, is prevented by nicotinamide(16). Manganese deficiency in the egg also produces a type of chondrodystrophy, but the tibiae are not curved and the embryos show marked edema and retarded down development. The failure of manganese, nicotina-

12. Landauer, W., and Dunn, L. C., PROC. SOC. EXP. BIOL. AND MED., 1926, v23, 562.

13. Landauer, W., *J. Exp. Zool.*, 1947, v105, 145.
14. Ancel, P., *Ann. d'Endocrinol.*, 1945, v6, 1.
15. Landauer, W., *J. Exp. Zool.*, 1948, v109, 283.
16. Zwilling, E., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 609.

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

* Present address: Denver General Hospital, Denver, Colo.

1. Riggs, L. K., *J. Am. Pharm. Assn.*, 1925, v14, 380.

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.