

ment, but after injections were located in the scrotum.⁶ Surgical means employed in 5 unilateral and 2 bilateral cases not responding to hormone treatment, showed adhesions or other mechanical factors preventing descent in all operated subjects. Penile erections, increased vascularity of the nasal mucosa, and growth of the scrotum, prostate and genital hair were observed. There was no lowering of vocal pitch. Twelve cases of pseudocryptorchidism treated with amounts of male hormone too small to give penile and scrotal growth were sufficient to condition the testes against spastic retention.

10070

Relation Between Size of Dose and Lipotropic Effect of Choline Chloride in Mice.*

MARY SCOTT WELCH AND ARNOLD DEM. WELCH. (Introduced by C. F. Cori.)

From the Department of Pharmacology, Washington University School of Medicine, St. Louis.

In recent years Best and his coworkers have established the effectiveness of choline chloride in preventing (or curing) fatty infiltration of the liver in depancreatized dogs and in rats on a low choline-high fat diet. Preliminary results with mice, chickens and puppies were reported by Best, Huntsman and Solandt,¹ but the choice of animals for choline studies has been restricted essentially to the rat and depancreatized dog.

We have found the mouse an excellent species for the study of the lipotropic action of choline chloride and related compounds, in many ways preferable to the rat. It is possible to complete an experiment satisfactorily in 5 or 6 days, while it has been the custom to conduct rat experiments for 2 to 4 weeks before sacrificing the animals. The reduction in the amount of low choline diet required and of difficultly prepared or expensive compounds related to choline chloride is important also.

⁶ Hamilton, J. B., *Endocrinology*, 1937, **21**, 649.

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¹ Best, C. H., Huntsman, M. E., and Solandt, O. M., *Trans. Roy. Soc. Canada*, 1932, Section V, 175.

Male mice of the same strain and of approximately the same age and weight (15-25 g) are arranged in groups of 8-12 in the experiments described and kept on a standard diet until the day previous to the beginning of the experiment. During the experimental period the diet consists of powdered beef muscle (Difco), well extracted with alcohol and ether, 5.3%; powdered sucrose, 48%; beef fat, 38%; cod liver oil, 2%; agar, 2%; salt mixture (U.S.P. XI No. 2), 4%; yeast concentrate,† 0.75%. The chemical to be tested is dissolved in a small amount of water and very thoroughly incorporated with the food; fresh supplies of the latter are placed daily in beakers (50 cc) which are wired to the cages; the weight of food residues is recorded daily. The animals waste little of their food supply when fed 2 g per mouse per day. The change in weight during a 6-day period may vary between $\pm 10\%$ but usually shows a small gain. The animals are decapitated and the livers analyzed for "total fat," which consists of the weight of the residue from the petroleum ether soluble fraction of the pooled livers, following saponification and acidification.

The efficacy of choline chloride in preventing deposition of "fat" in the livers of the mice may be expressed as follows: $\frac{x-y}{x-3.5} \cdot 100 =$ percentage effectiveness of test dose; where x represents the percentage of "fat" in the livers of control animals; y , the percentage of "fat" in the livers of animals which received choline chloride and 3.5 the average percentage of "fat" in the livers of normal mice.

In Fig. 1 A the results of such calculations are plotted against

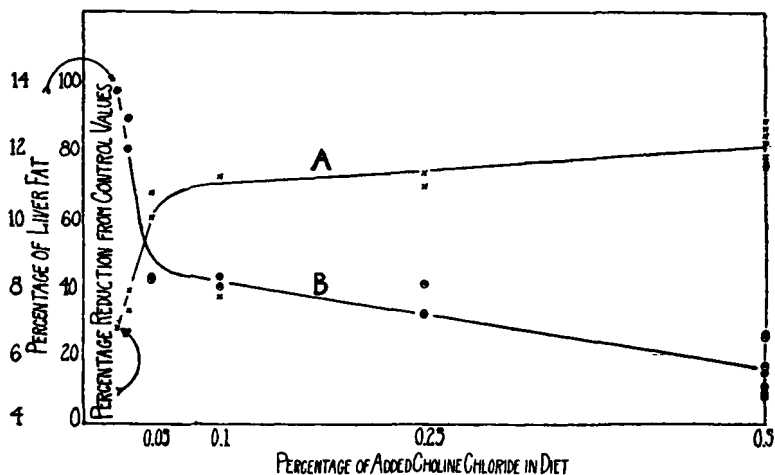


FIG. 1.

† Generously supplied by Anheuser-Busch, Inc.

dosage of choline chloride. In Fig. 1 B the actual percentage of liver fat in the test animals, without reference to the value in the controls, is plotted against choline chloride dosage.

The curves indicate that at the higher dosage levels the effect of increasing the concentration of choline chloride in the diet is slight and only at levels of less than 0.05%, under the conditions described, is there a marked correlation between concentration and action. It would appear evident that in assaying the activity of any lipotropic substance it is important to use relatively small doses in order to obtain the most significant relation between the size of the dose and the lipotropic action produced.

It will be appreciated that under any conditions the amount of "extra fat" actually "transported" or otherwise disposed of due to the influence of added choline chloride is relatively small. A 20 g mouse under the experimental conditions will deposit only about 0.15 ± 0.05 g of "extra fat" in the liver in the absence of added choline chloride, although consuming nearly 5 g of fat during the 6-day test period. From the figures it is evident that the smaller the dose of choline chloride the greater is its relative efficiency in preventing the deposition of a portion of this small amount of fat, that is, more fat is "transported" per molecule of choline chloride.

Summary. A 6-day method for the assay of lipotropic substances in the mouse is described. It is shown that a significant relation between the concentration of choline chloride added to a low choline-high fat diet and the lipotropic effect produced exists only at levels approaching the minimum effective dose, under the experimental conditions described.

10071 P

Lipotropic Action of Certain Compounds Related to Choline Chloride.*

ARNOLD DEM. WELCH AND MARY SCOTT WELCH. (Introduced by C. F. Cori.)

From the Department of Pharmacology, Washington University School of Medicine, St. Louis.

The hypothesis that choline chloride functions lipotropically by

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