ON THE NUTRITIVE STUDIES OF PANTOTHENIC ACID BY ANTIMETABOLITES II. EFFECT OF ω-METHYL PANTOTHENIC ACID ON THE DEVELOPMENT OF THE CHICK EMBRYO

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II. EFFECT OF ω-METHYL PANTOTHENIC ACID ON THE DEVELOPMENT OF THE CHICK EMBRYO

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Effects of maternal diet on the embryonic development of chicken have been the subject of numerous investigations up to the present. Such studies, however, have been proposed for determining the quantity of a nutritional factor necessary to support the optimum hatchability and subsequent livability of the offspring. Thus a considerable number of results have been presented which show the effect of a dietary deficiency as an increase of embryonic mortality or/and as the abnormalities of the growing embryo. However, such experiments have given little information to the understanding of the metabolic process of the developing chick embryo, even though they have made a great contribution in the field of poultry industry.

Generally speaking, the analysis by utilizing vitamin antagonists have been extensive. In spite of the fact that the use of antimetabolites in analysing metabolic processes is fruitful, their use in the field of chemical embryology has been limited. Along this line, the experiments appeared since 1949 in determining the essential vitamins for the developing chick embryo. Cravens and Snell (1) were the first who studied the effects of desoxy-pyridoxine on the development of the chick embryo. Kranofsky *et al.* (2) examined the folic acid metabolism in the same manner. These two works are impressive, the experiments applying the inhibition technique may be helpful in determing the functions of vitamin in embryonic development.

Drell and Dunn (3, 4) reported that ω-methyl pantothenic acid is an antagonist of pantothenic acid for many species of lactobacilli and, later, for rats and mice. The confirmation of the fact in mice was also succesful in our laboratory as had been preliminary described experimentally (5). Gillis, Heuser and Norris (6) found that when hens were fed with a diet of ordinary feedstuffs heated to destroy pantothenic acid, their eggs failed to hatch unless

this factor was supplemented. Similar results have been reported by Kratzer *et al.* (7).

In the present study, the possibility to induce a nutritional deficiency of chick embryos by injecting ω-methyl pantothenic acid was studied.

Materials and Methods

ω-Methyl pantothenic acid used in these studies was prepared by the method as described by Drell *et al.* This analogue had been found to give an inhibition index of 500 against pantothenic acid with *L. arabinosus* 17–5. Eggs from white Leghorn hens were used throughout the study. They were incubated at the temperature of 38°C and antimetabolite was injected on the second day. For injection the blunt end of the egg was cleaned with 80 per cent ethanol, and a small hole was drilled through the shell. Injections were made into the yolk sac with a 1 cc tuberculin syringe fitted with a sterile needle. The opening was then sealed with Scotch tape. A series of preliminary tests were carried out to determine the concentration of ω-methyl pantothenic acid effective in imparing the embryo viability. All injected solutions were prepared to contain 0, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, and 30.0 mg ω-methyl pantothenic acid in 0.1 *ml* of water. Eggs were candled every fourth day for viability. With the former seven concentrations no apparent effect was shown in viability of embryos and hatchability. The latter three concentrations almost killed the eggs as will be described in the text. Accordingly the 10 mg dosage was used in the subsequent study for determining the interference ratio against pantothenic acid.

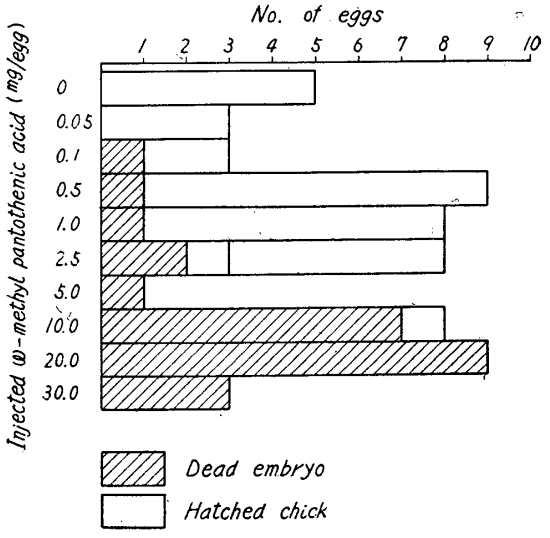

Fig. 1. Effect of injection of ω-methyl pantothenic acid upon chick hatchability. (Injection on the second day of incubation)

Results

Fig. 1 illustrates that the injection with more than 10.0 mg of ω-methyl pantothenic acid on the second day of incubation causes 100 per cent death of the embryos. Among them 80 per cent of embryos succumbed within the first week. The injection was also made in eight days' old embryo, but almost all of tested eggs survived, suggesting that the lethal dose of ω-methyl pantothenic acid into eight days' old embryo increased more than 30.0 mg. Subsequent experiment showed the

inhibitory effect of the analogue was markedly prevented by the simultaneous injection of 0.05 mg or more of pantothenic acid as shown in Fig. 2. From these figures, the interference ratio was roughly calculated as 200 : 1.

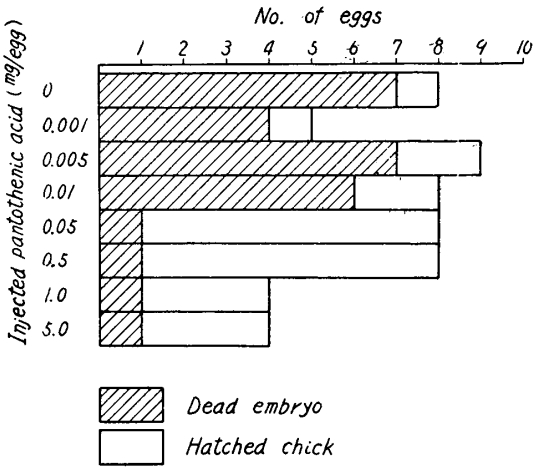

Fig. 2. Effect of simultaneous injection of pantothenic acid to lethal dose (10.0mg/egg) of ω-methyl pantothenic acid.

Discussion

Kratzer *et al.* found that hatchability was depressed by pantothenic acid deficiency in the breeder diet and they reported malformations of the legs in deficient embryos. Zunin and Borron (8) reported a dehiscence of the cerebral matter in newborn rats after the injection of the female parents with pantoyltaurine. Goetinck *et al.* (9) observed the same symptoms in turkey embryos, and suggested that ω-methyl pantothenic acid was effective in inducing pantothenic acid deficiency symptoms.

In the present study we have found that ω-methyl pantothenic acid caused the death of the two days' old chick embryo and the toxic effect was prevented by simultaneous dose of pantothenic acid. These results may suggest that the analogue cause a pantothenic acid deficiency in the chick embryo as was the case in the mice.

Moreover, we have an unrecorded finding where these survived chicken after the injection of ω-methyl pantothenic acid tend to decrease the Coenzyme A content in their liver. In the previous studies (10), we presented the fact that ω-methyl pantethine interrupted the acetylation of sulfanilamide but with regard to the same effect ω-methyl pantothenic acid failed to do so. These results suggest the mode of mechanism of interfering action of ω-methyl pantothenic acid. The possible synthesis of ω-methyl pantetheine in vivo from ω-methyl pantothenic acid have been considered to explain the fact here described.

Summary

1. Injection with more than 10 mg of ω-methyl pantothenic acid on the second day of incubation causes almost 100 per cent death of the embryos.

2. A lethal dose of ω-methyl pantothenic acid on the second day of incubation was 10.0 mg per egg and 80 per cent of the embryos died within the first week.

3. Inhibitory effect of ω-methyl pantothenic acid was markedly prevented by the simultaneous injection of pantothenic acid. Interference ratio was 200 : 1.

4. The mechanism of interference action of ω-methyl pantothenic acid was discussed.

Acknowledgement

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References

1) Cravens, W. W. and E. E. Snell, (1949). Proc. Soc. Exp. Biol. Med., **71**, 73.
2) Kranofsky, D. A., P. A. Patterson and L. P. Ridgeway (1949). Ibid., **71**, 447.
3) Drell, W. and M. S. Dunn (1946). J. Amer. Chem. Soc., **68**, 1868.
4) Drell, W. and M. S. Dunn (1948). Ibid., **70**, 2057.
5) Kimura, S. and H. Ariyama (1959). (to be published)
6) Gillis, M. B., G. H. Heuser, and L. C. Norris (1942). J. Nutrition, **23**, 153.
7) Kratzer, F. H., P. N. Davis, B. J. Marshall and D. E. Williams (1955). Poultry Sci., **34**, 68.
8) Zunin, C. and C. Borron (1954). Acta. Vitaminol., **8**, 263.
9) Goetinck, P.F., U. K. Abbott and F. H. Kratzer (1957). Poultry Sci., **36**, 455.
10) Unpublished data.