IV. THE EFFECT OF CHEMICAL PRESERVATION OF EGGS UPON THE STABILITY OF THEIR VITAMIN CONTENTS.

BY ERNEST TSO.

*From the Department of Medicine, Peking Union Medical College,
Peking, China.*

(Received November 16th, 1925.)

CHINESE preserved eggs, or "pidan," offer an unusual opportunity for the study of some of the conditions under which the stability of vitamins may be affected. The preserved eggs are more or less chemically changed products of fresh ducks' eggs which are presumably rich in vitamin A, vitamin B, and the anti-rachitic food factor which exhibits certain vitamin-like properties. It should therefore be of interest to know what effects the preserving agents and the chemical changes have upon the stability of these vitamins. Furthermore, such a study would at the same time disclose the vitamin value of "pidan" as a food. This is of practical significance in China where the preserved egg is perhaps as much used on the table as is cheese in Western countries.

The method of preservation according to the Chinese Economic Bulletin is the following.

Fresh ducks' eggs are selected and washed. For the preservation of 100 eggs, mix 5 ounces of pure soda, 25 ounces of straw ash, and a little less than 4 ounces of table salt with about 20 ounces of boiling water to give a uniform mixture. Then add gradually 40 ounces of slaked lime to make a thick paste. Apply heat if necessary and keep stirring until the ingredients are thoroughly mixed. A layer of this mixture about a quarter of an inch thick is wrapped round each egg and covered with rice husks to prevent sticking. The eggs are then carefully laid in earthenware jars which are sealed with wet clay when full. In about a month the eggs acquire the desired flavour and degree of coagulation and are ready for the table.

"Pidan" looks quite different from the fresh egg. The white has not only coagulated but turned dark brown and translucent, not unlike coffee jelly in appearance. The coagulated yolk looks deep green or greenish grey with concentric rings of different shades of grey, brown or dark green. The egg has a peculiar piquant lime taste and a decidedly ammoniacal odour, especially when freshly opened.

Blunt and Wang [1917] some time ago analysed these eggs and found that there is a marked increase in the ash content and in the alkalinity of the ash, and a partial decomposition of the proteins and phospholipins resulting in an excessive production of free ammonia and in a diminution of the yolk-fat. According to these authors, the characteristic changes in the eggs are brought about by the combined action of bacteria and enzymes, as well as by the alkaline preservative.

EXPERIMENTAL.

I. Vitamin A.

Exp. 1. In a preliminary test the results of which are presented in Fig. 1, it was found that the addition of 20 % of the preserved egg-yolk to a basal ration deficient in vitamin A, on which four young rats had declined in weight and developed xerophthalmia, induced promptly the return of vigorous growth and the cure of the eye condition. The two controls continued to decline.

Fig.1. Ration consisted of caseinogen 18, corn-starch 72, yeast 5, and salt mixture 5. At the position of Y the amount of yeast was doubled. Arrowhead indicates the addition of 20 % of the yolk of the Chinese preserved egg, and double arrowhead the withdrawal of the yolk.

Exp. 2. Eight albino rats 4 weeks old weighing on the average 43 g. were placed on a basal ration of caseinogen 20, corn-starch 65, yeast 10, and McCollum's 185 salt mixture 5. The caseinogen, starch, and yeast had been purified by oxidation and tested to be free from vitamin A but satisfactory in other respects.

After the initial growth, which lasted from 6 to 8 weeks, all animals except one ceased to gain and towards the end of the 8th week three developed xerophthalmia. At this point half of the animals, including one with sore eyes, were put on the basal ration plus 5 % of the preserved egg-yolk, incorporated into the basal ration paste. The other four rats were placed in a similar manner on 5 % of liquid yolk from fresh ducks' eggs. The feeding was continued for

4 weeks. The results are shown in Figs. 2 and 3 respectively, from which it will be seen that there was an immediate response to the addition of the preserved yolk as well as the fresh yolk as indicated by the prompt return of vigorous growth and the cure of the eye condition in both groups of animals.

Exp. 3. Four young rats, which had declined in weight on the basal ration deficient in vitamin A, were fed 3 % of the preserved egg-yolk. The response was quite as definite though not so immediately apparent as by adding 5 % of the preserved yolk.

Fig. 2. Diet consisted of caseinogen 20, corn-starch 65, yeast 10, and salt mixture 5. At the point of arrowhead 5 % of preserved egg-yolk was added.

Fig. 3. Ration as in Fig. 2. Arrowhead indicates the addition of 5 % of liquid yolk from fresh ducks' eggs.

Exp. 4. Six rats 4 weeks old were placed on the same basal ration as used in previous experiments. In the 8th week, when growth in most of the animals had ceased, three were given, in addition to the basal ration, 2 % of oxidised ether extract of dried preserved egg-yolk¹. In the 10th week the other three rats received, in addition to the basal ration, 2 % of the same lot of ether extract, but not oxidised. The experiment was terminated at the end of 12 weeks of feeding. The results are presented in Fig. 4, from which it will be seen that those rats receiving the oxidised ether extract from the beginning of the 9th week continued to lose weight and developed xerophthalmia, and one

¹ The preserved egg-yolk had been dried in an oven at 45° for three days and next extracted with ether in a Soxhlet, the ether extract had then been exposed to an air-bath at about 120° for six hours.

died before the 12th week. On the other hand, the other three animals immediately responded to the addition of 2 % of non-oxidised ether extract as shown by the return of good growth and the improvement in general well-being of the animals.

From the results of the foregoing experiments we may conclude that the chemical changes occurring in the Chinese preserved eggs have little or no deleterious effect upon the stability of vitamin A.

Fig. 4. Diet consisted of caseinogen 20, starch 65, yeast 10, and salt mixture 5. At the position of single arrowhead 2 % of the ether extract of preserved egg-yolk was added. Double arrowhead indicates the addition of 2 % of oxidised ether extract.

Fig. 5. Diet consisted of caseinogen 18, corn-starch 67, fresh butter-fat 10, and salt mixture 5. At the point of arrowhead 25 % of the preserved egg-yolk was added. During the 12-hour period marked between the double arrowheads, 10 g. of liquid yolk from fresh ducks' eggs was fed.

II. Vitamin B.

Exp. 1. Six young rats which had grown vigorously on a normal diet were transferred to the following basal ration deficient in vitamin B: caseinogen 18, corn-starch 67, fresh butter-fat 10, and McCollum's 185 salt mixture 5. Beginning in the 4th week when all the rats had declined in weight, three were fed, in addition to the basal ration, 25 % of the preserved egg-yolk. As illustrated in Fig. 5, these three rats continued to lose ground and two died about 3 weeks later. The third rat would also have succumbed but for a change made in its diet. This consisted of a single feeding of about 10 g. of fresh ducks' egg-yolk. Over-night the animal gained 34 g. and began to eat more than usual amounts of the preserved yolk and basal ration mixture. During the following 9 days another increment of 50 g. in weight was added. The three control rats on the basal diet alone lost weight rapidly after the initial growth of 1 to 3 weeks' duration. One died in the 5th week, another in the 6th, and the third in the 7th week.

This experiment indicates that the chemical changes in the preserved eggs are quite unfavourable to the stability of the growth-promoting vitamin B. Apparently it is the presence of alkaline products in the preserved eggs that is destructive of the originally rich content of vitamin B in the fresh ducks' eggs, since it is a well-known fact that vitamin B is very sensitive to alkaline reagents.

III. *The anti-rachitic food factor.*

Exp. 1. Sixteen albino rats 4 weeks old were placed on Sherman and Pappenheimer's 84 basal rachitic diet, which consists of patent flour 95, calcium lactate 2.9, sodium chloride 2, and ferric citrate 0.1. At the end of 3 weeks when the animals should all have developed rachitic lesions in the bones they were divided into 4 groups of 4 each. To the basal ration of one group was added 5 % of the preserved egg-yolk, of another group 5 % of oxidised preserved yolk, of a third group 5 % of fresh ducks' egg-yolk, and of a fourth group 5 % of olive oil. Two weeks later the animals were killed under ether and X-rayed. The X-ray examination showed that healing of rachitic bone lesions had occurred in the rats which received either the fresh yolk, the preserved yolk, or the oxidised yolk. Healing was, however, most pronounced in the rats receiving fresh yolk and least marked in the animals fed the oxidised preserved yolk. The control rats taking olive oil all showed rachitic lesions of a severe grade without presenting any evidence of healing.

Exp. 2. In this experiment the anti-rachitic property of the ether extract and the oxidised ether extract of preserved yolk was tested. This ether extract had been found to contain vitamin A but the oxidised product to be free from vitamin A. The results and other details are presented in Table I.

Table I. *The anti-rachitic potency of the ether extract from the yolk of Chinese preserved eggs.*

Ration	Pre-experiment diet		Age of rats Days	Duration of feeding Weeks	Initial wt. g.	Final wt. g.	Results	
	Milk Butter	Soybean Millet Corn Cabbage					X-ray examination	Microscopic examination
1. Basal plus 2 % ether extract	R. 378	—	24	3	40	52	No rickets	No rickets
	R. 379	—	24	3	30	37	„	„
	—	R. 382	30	3	40	62	„	„
2. Basal plus 2 % oxidised ether extract	R. 376	—	24	3	34	42	? Slight rickets	No rickets
	R. 377	—	24	3	34	43	„	„
	—	R. 381	30	3	42	60	Rickets	Slight rickets
3. Basal	R. 380	—	24	3	34	42	Rickets	Rickets
	—	R. 383	30	3	33	44	Severe rickets	Severe rickets
	—	R. 384	30	3	44	58	„	„

These experiments show that the anti-rachitic food factor like the growth-promoting and anti-xerophthalmic vitamin or vitamin A is not affected to any appreciable extent by the chemical changes in the preserved eggs. That the oxidised ether extract was found to be less effective in preventing rachitic

bone lesions might be due to factors other than oxidation, since it has been shown by Hess and Weinstock [1924] that even drying the yolk (of hens' eggs) and keeping it in a dried state would markedly reduce its anti-rachitic value.

SUMMARY AND CONCLUSION.

"Pidan" is made from raw ducks' eggs by applying a mixture of slaked lime, straw ash, soda, table salt, and water.

A study of the stability of the vitamins in these preserved eggs has shown that the originally rich vitamin B content is practically completely destroyed but that the potency of vitamin A and the anti-rachitic food factor is little or not at all affected.

REFERENCES.

- Blunt and Wang (1917). *J. Biol. Chem.* **28**, 127.
Hess and Weinstock (1924). *Proc. Soc. Exp. Biol. Med.* **21**, 441.