DEMONSTRATION OF FOOD PROTEINS IN HUMAN BREAST MILK BY ANAPHYLACTIC EXPERI- MENTS ON GUINEA-PIGS

THEIR PROBABLE RELATIONSHIP TO CERTAIN DISEASES
OF THE NURSING INFANT *

A PRELIMINARY REPORT

W. RAY SHANNON, M.S., M.D.

Associate in the Department of Pediatrics, Miller Hospital Clinic

ST. PAUL

Our forefathers believed that foods ingested by the nursing mother might affect the child through the breast milk. We, however, have discarded that belief almost universally, and have relegated it to the shelves of superstition simply because we have not succeeded in proving it to be true. Having seen several cases of food disturbance in infants which it seemed could have come only through breast milk, I determined to investigate this subject to see if I could prove by anaphylactic tests that such was the case. It is the purpose of this paper to record the results of this investigation.

Generally speaking, the subject of diet for the nursing mother is passed over lightly. The most widely accepted view at the present time is that she may eat whatever agrees with her. "The mother may eat whatever is good for the father," is a very common expression. Pfaundler and Schlossman's textbook¹ contains the statement that "a nursing mother may eat whatever she pleases." Abt² says, "Nearly every writer on subjects of maternal nursing has declared against the view that certain foods ingested by the mother are capable of producing disturbance in the infant through the breast milk." These statements unquestionably crystalize the general sentiment of those interested in the subject of maternal nursing today.³ Restrictions, if there be any, are made for fear unrestricted diet might result in intestinal upsets in the mother and not because it is thought that harm might come to the

* Received for publication, April 25, 1921.

* Published from the Miller Hospital Clinic.

1. Pfaundler and Schlossman: English translation 1:356, 1912.

2. Abt: Facts and Fallacies About Breast Feeding, St. Paul M. J. p. 281, (April) 1915.

3. Foote, J.: Washington M. Ann. 16:82 (March) 1917. Durand, J. I.: Northwest Med. 15:89 (March) 1916. Greer, B. E.: Texas State J. M. 13:213 (Oct.) 1917.

infant through the breast milk. Thus Holt ⁴ makes mention of the fact that salads and highly seasoned foods should be avoided, not so much because they upset the child as because they are apt to upset the digestion of the nurse.

While this seems to be the prevailing opinion, the literature is not devoid of warnings that blind adherence to such a principle might, in certain cases, prove disastrous.⁵ Thus Rotch,⁶ in 1901, says "Certain vegetables and sometimes fish will, in individual cases, affect the milk and cause discomfort to the baby." Griffith ⁷ also states that in exceptional cases, where the mother may have idiosyncrasies to certain foods, the eating of such foods may affect the breast milk so as to cause disturbance to the infant. Colburn ⁸ makes a similar statement.

The preceding statements, apparently, have been largely in the nature of impressions, and have not been followed up by any convincing proof. Talbot,⁹ however, mentions a case in which a very severe eczema cleared up on the mother's discontinuing the eating of chocolate and recurred on her again eating that food.

The most convincing arguments for the transmission of foods eaten by the mother to the infant through the breast milk are presented by E. S. O'Keefe.¹⁰ He found that some of his nursing infants with eczema gave positive skin reactions to proteins they had never eaten. One infant was susceptible to cow's milk casein, another to egg and oats, still another to codfish, and several others to egg protein. In some of the cases, recovery was prompt on the removal of the offending protein from the mother's dietary, while in other cases it was slow. Still other cases showed no improvement at all. The author suggests that a biologic investigation might be of interest to ascertain whether or not the protein of the foods could be identified in the breast milk.

During the past winter the following case came under my care.

REPORT OF CASE

A male infant, 7 months old, was brought in by the mother because of a profuse skin eruption, involving practically the entire body. This eruption had first been noticed about two weeks previously. It first appeared on the backs of the hands in the form of groups of small papules resembling mosquito bites. From the backs of the hands it had spread to the forearms, arms, face and chest, and finally over the entire body, being most marked on the extremities. The papules in many cases would turn into vesicles which, when rubbed off by scratching, became covered with crusts. Some became filled with pus, probably due to secondary infection.

4. Holt: *Diseases of Children*, 1917, p. 142.

5. Mulherin: *J. A. M. A.* **75**:855 (Sept. 20) 1920. (See discussion by Dr. Chas. J. Bloom). Westcott: *Am. J. Obst.* **78**:55.

6. Rotch: *Pediatrics*, 1901, p. 137.

7. Griffith: *Diseases of Infants and Children*, 1919 **1**:105.

8. Colburn, W. B.: *Nebraska State M. J.* **4**:243 (Aug.) 1919.

9. Talbot, F. B.: *Med. Clin. N. America*, **1**:985 (Jan.) 1918.

10. O'Keefe, E. S.: *Boston M. & S.* **182**:569 (Nov. 11) 1920.

Past History.—With the exception of a few points the past history was negative. The feeding had been irrational, the baby having been fed every time he cried. Off and on, of late, the mother had been giving cream of wheat, oatmeal, cow's milk, cream, potatoes, tea and some beef, apparently helping the child from the family table. In spite of this the child had thrived, being somewhat overweight and having had no illnesses, more than occasionally frequent stools and a few colds, until the onset of the present illness.

Physical Examination.—Aside from the skin condition the physical examination was essentially negative. Over the entire body was a profuse papular eruption. This was very marked over the backs of the hands and fingers, forearms, scalp and neck. The papules were largely confluent over these areas, giving them a thickened, swollen appearance. Over the trunk, thighs, legs, feet, the upper arms and face the lesions were less numerous. One patch only was present on the back and this was in the lower left lumbar region. The eruption was present also on the ears, palms and soles, and on the penis and scrotum. There were no lesions in the mouth.

The Papules.—In size, the papules varied from pin-head to one-half the size of a bean. The majority of papules were capped by vesicles which also varied in size from pin-point, topping the papules, to one-half the size of a bean and replacing it entirely. The largest vesicles were seen where the eruption was thickest and on the palms and soles. All vesicles were tense and filled with fluid of varying degree of turbidity. The larger ones were thick walled.

In the old lesions, the papules were dark red in color. Frequently, here, the vesicles had been replaced by black crusts. Lesions of this type were scattered over the entire body. In the scalp a few lesions were infected, and presented the appearance typical of impetigo contagiosum, probably due to secondary infection.

The early papules were whitish or pinkish in color and often appeared in clusters or groups. They were found interspersed with the older lesions over the entire body. They seemed to itch moderately, though not so severely as some forms of eczema. To palpation they felt shotty, similar to the early lesions of smallpox, which some of the papules strongly resembled.

Diagnosis.—Apparently the disease was in the nature of a chronic urticaria, and probably due to some food eaten by the patient. Therefore, the child was taken off all artificial foods, and local applications were applied for a period of one week with no improvement. In fact, the eruption became more profuse. It then seemed advisable to place the child on a hospital regime and to attempt to find the underlying cause. This was done.

Treatment.—After a study of the diets of mother and patient the latter was tested by the cutaneous method with the following proteins; cow's milk casein, egg white, egg yolk, banana, oats, potato, corn, beef, wheat, peas, rice, barley, carrots, tomato and pork. Definitely positive reactions were obtained to egg white, egg yolk and oats. Questionable reactions were obtained in the cases of banana, wheat, peas and tomato. The mother asserted that the baby had never received eggs, bananas, peas or tomato in his diet. The mother was instructed to eat no egg or food containing egg, oats, banana, wheat, peas, rice or tomato, and the baby was held to a diet of breast milk alone.

Clinical Course.—Improvement was very rapid so that in three days all the lesions had lost their ruddy color and were covered by thin colorless crusts. No new lesions had appeared. This improvement continued till the fifth day when the mother thought the infant so well recovered that she signed him out against advice.

Soon after getting the child home the mother began giving him crusts of bread. She herself began eating wheat bread toast for breakfast. On the third day after leaving the hospital new lesions began to appear. The mother lost heart in the matter of following directions and the condition of the skin reverted to its former state, although to a less degree.

Comment.—It seemed that the breast milk must be responsible in some way for the eruption, most probably by carrying the proteins to which the infant was susceptible. An analytical investigation by means of anaphylactic reactions on guinea-pigs was, therefore, undertaken in an attempt to prove the presence of some of these proteins in the breast milk after they were eaten by the mother.

Before proceeding with the experimental work, it might be well to discuss, briefly, the underlying principles on which the investigation was based.

Anaphylaxis is a phenomenon occurring in the animal body, or under certain conditions in the excised parts, as a result of the introduction of a foreign protein after the animal has been sensitized to that protein. Sensitization may be inherited, passively acquired, or actively attained. The latter form results within a variable period of time, usually in at least from eight to ten days, after the protein substance is introduced parenterally into the body. Such introduction may be through the intestinal mucosa by the absorption of undigested protein, by intravenous, intraperitoneal or subcutaneous injection, and probably also by absorption through any of the mucous membranes of the body. In experimental work, the intraperitoneal injection is probably the method of choice and was used in this work.

The degree of sensitization is affected in various ways by the initial or sensitizing dose. It has been demonstrated that small initial doses give rise to sensitization more quickly than large doses, although the ultimate degree of sensitization in both cases may be the same.¹¹ It has further been demonstrated that a primary dose sufficiently large to produce sensitization when injected alone, may totally fail to do so when injected together with or twenty-four hours after a large amount of some other protein.¹²

It is necessary that the second or shock dose be of the same type of protein as the sensitizing dose. In this regard the reaction is highly specific.¹³ Wells says, "the anaphylaxis reaction exhibits exactly the same degree of specificity that we obtain with complement fixation or precipitin reaction." A guinea-pig sensitized to egg protein will exhibit anaphylaxis only on the subsequent injection of egg protein, one sensitized to horse serum requires horse serum for the production of shock, etc. This specificity is absolute for such widely different proteins as those cited, and the fact that group reactions are sometimes obtained when like proteins of closely related species are used need not be considered in the work herein reported.

11. Besredka: *Anaphylaxis and Anti-anaphylaxis*. English translation, 1919.

12. Lewis: *J. Infect. Dis.* **17**:241, 1915. (Quoted from Wells).

13. Wells, H. G.: *Physiol. Rev.* **1**:44. Von Pirquet: *Arch. Int. Med.* **7**:259, 383 (Feb. March) 1911. Boughton, T. H.: *J. Lab. & Clin. M.* **5**:97 (June) 1920.

The route by which the second dose is introduced into the body is of great importance. According to Besredka and others, intravenous injection gives rise to the most delicate test. However, it has the disadvantage that it may give rise to false reactions because of embolism, blood coagulation, etc. The next most delicate test in the intact animal is induced by the intracerebral injection. Introduction of the shock dose by this route has been demonstrated by Besredka to be many times more delicate than the intraperitoneal or subcutaneous routes. The intracerebral injection is, therefore, the method of choice and was used in this work. The uterine strip method may also be used.

Animals vary considerably in their apparent susceptibility to and in the symptomatology of anaphylactic shock. While the degree of susceptibility varies among different individuals of the same species, the symptomatology is fairly uniform for a given type of animal. Therefore, it is necessary, in comparative work, to use the same species of animal throughout. The most characteristic symptomatology is seen in guinea-pigs and thus these animals are used by the majority of observers in anaphylactic experiments.

The characteristics of anaphylactic shock as seen in the guinea-pig are outlined by Besredka as follows:

Scarcely is the test injection terminated than the animal begins to struggle; it scratches its muzzle as if wanting to remove a foreign body. Its struggles become more and more marked; it commences to turn round and round and then to turn somersaults which become more and more frequent and violent. Then these convulsive attacks begin to take place at greater intervals, and the animal having become weakened from the output of so much energy, lies on its side. The vesicle and anal sphincters become relaxed; there is involuntary discharge of urine and feces. The respiratory movement, dyspneic at first, become slower and slower and finally become paralyzed, and at the end of an interval which varies from three to seven minutes, the animal dies of asphyxia.

This is, of course, the characteristic fatal shock. It does not necessarily follow that all tests must conform entirely to this description to be classed as anaphylaxis.

It will readily be seen from this brief exposition of the subject of anaphylaxis that it leads itself readily to the identification of the proteins in the breast milk. It was with this fact in mind that the following experiments were carried out.

PROTOCOLS OF EXPERIMENTS

Experiment 1.—Breast milk obtained from the mother of Case 1, after she had been eating no eggs for a period of ten days was injected into each of three guinea-pigs as a sensitizing dose. Two weeks later two of them were injected intraperitoneally with a solution of egg white and yolk. The third was injected with a few drops after the manner of Besredka.

Nothing unusual occurred in any case.

Experiment 2.—The mother was instructed to eat two eggs for breakfast on three successive days. Breast milk was obtained on each of these days at noon and a portion of each sample was injected into each of three pigs. A portion of the last sample was taken to Dr. Larson for preservation under pressure.¹⁴

Twelve days later two guinea-pigs were injected intraperitoneally with a mixture of egg white and yolk. The third guinea-pig was injected intrathecally with a few drops of the same mixture.

Aside from immediate signs of irritability nothing appeared in the guinea-pigs receiving intraperitoneal injections.

The third guinea-pig showed immediate nervousness, followed by spasmodic so-called bucking movement, shivering, chattering of teeth and partial paralysis of the hind legs. Gradual recovery inside of half an hour.

Experiment 3.—Three guinea-pigs were injected with 1 c.c. of a mixture of egg white and yolk. Twelve days later two guinea-pigs were injected intraperitoneally with 3 c.c. of the breast milk used in Experiment 2, which had been preserved by Dr. Larson. The third guinea-pig was injected intrathecally with a few drops of the same sample.

In the first two pigs nothing more definite than a severe dyspnea and marked nervous irritability were noted.

The third guinea-pig immediately fell over, kicking all four legs. Got up, hopping up and down. Extreme dyspnea was present. Fell over and got up repeatedly, each time with considerable difficulty. Definite paralysis of the hind legs noted twenty minutes after injection. Chattering of teeth, frequent defecation, marked dyspnea occurred together with the above symptoms for a period of one hour when the animal seemed to recover somewhat. Found dead seven hours after injection with body still warm.

Controls: This experiment was duplicated on three control guinea-pigs, none of which had been sensitized to egg protein. Nothing resulted.

Experiment 4 (Repetition of Experiment 3).—Mother was instructed to eat six eggs in one day—two at each meal. Milk obtained at 5 p. m. and before the 6 a. m. nursing the next day. Specimen mixed and given to Dr. Larson for preservation. At the same time two guinea-pigs were sensitized to egg white and yolk.

Twelve days later each guinea-pig was injected intrathecally with a few drops of the preserved milk.

The first guinea-pig showed immediate nervousness and dyspnea. In five minutes it fell over, kicking all four legs but rapidly got on its feet. Transitory paralysis of one hind leg. Defecated and urinated frequently. Recovery from most marked symptoms in fifteen minutes, but continued to be very irritable and to defecate frequently for a period of one hour. Normal in the morning and has been so since.

The second guinea-pig had immediate convulsion. Fell on side and kicked vigorously with head held in extreme retraction. Marked dyspnea. This continued without change for a period of twenty minutes. Then seemed to recover somewhat and got to feet very unsteadily. After this was up and down for a period of one hour at the end of which time there was still noted some paralysis of the hind legs. Five hours later there was still some paralysis of the hind legs but less marked. Perfectly normal in the morning and has been so since.

Control: A nonsensitized pig was injected intrathecally with the same amount of the same specimen of milk. Nothing unusual occurred.

CASE 2.—This case is of interest. A baby boy, aged 6 weeks, seen because of crying, especially at night, had frequent curdy stools and gaseous distension of the abdomen.

The baby is the second child of young and healthy parents. Breast fed entirely every four hours five times a day. He got along well for the first few

14. Larson et al.: J Infect. Dis. **22**:271 (March) 1918.

weeks, gaining very rapidly. The mother had an abundance of breast milk, which seemed to be very rich from its appearance on standing. An analysis of a twenty-four hour sample of milk taken before, in the middle of and after feeding for the five feedings of one day, had shown 6½ per cent. fat.

The mother had already been advised to put the baby on an amount of breast milk which was about the optimum amount, figuring 100 calories to the kilogram. In spite of this, the colic and frequent stools had not cleared up, although the infant had gained about normally.

A few days prior to my seeing the baby a fine papular rash had begun to appear over the forehead, cheeks and scalp. This seemed to itch tremendously and the infant scratched incessantly. The lesion was typical of beginning eczema and having my previous experience fresh in mind I inquired into the diet of the mother. I ascertained that she was eating one egg daily.

Experiment 5.—Two guinea-pigs that had previously been sensitized to egg white and yolk were given intrathecal injections of a few drops of the fresh breast milk.

The first guinea-pig showed immediate irritability, extreme dyspnea, and convulsive hopping movements which continued for a period of thirty-five minutes. At the end of this time, 1 c.c. of the milk was injected intraperitoneally. The animal died within twenty-four hours.

The second guinea-pig immediately fell over in a convulsion. It lay on its side and kicked all legs vigorously, at the same time showing extreme dyspnea. This lasted twenty minutes. At the end of this time the animal recovered somewhat and tried to get on its feet, but it fell back. This continued for fifteen minutes more when the animal was injected intraperitoneally with 1 c.c. of the same breast milk. The animal died some time within the twelve hour period following.

Control: A nonsensitized guinea-pig was injected similarly with a few drops intrathecally, followed thirty-five minutes later by 1 c.c. intraperitoneally. Nothing unusual was noted in either case, and the guinea-pig was still alive at the end of eight days.

Experiment 6.—The mother was taken off eggs and two weeks later a guinea-pig which had been sensitized to egg white and yolk was injected intrathecally with a few drops of breast milk obtained fresh at that time. Nothing occurred.

CASE 3.—This was an infant showing, in addition to the signs of over-feeding, a papular, itchy eruption over the cheeks. The mother had been eating two to three eggs daily so that a sample of breast milk was obtained and the following experiment was conducted.

Experiment 7.—The sample of milk obtained, while the mother was on a diet containing two or three eggs daily, was given to Dr. Larson for preservation. At the same time, two guinea-pigs were sensitized to a solution of egg white and egg yolk. Two weeks later these guinea-pigs were injected intrathecally with a few drops of the preserved milk.

The first guinea-pig became immediately extremely irritable, hopping up and down, showing marked dyspnea and rubbing face with front paws. Frequent defecation. Recovery from the worst nervous signs in fifteen minutes, although continued to defecate frequently and to appear sick for a period of one hour. At the end of this time it was injected intraperitoneally with 1 c.c. of the same solution. The guinea-pig died during the night.

The second guinea-pig showed immediate nervousness. Began moving round and round in circles. Marked dyspnea. Fell on side fifteen minutes after injection. Got up unsteadily. Five minutes later rolled over and over across the floor. Then got to feet, staggering markedly, especially in the hind quarters. These signs continued for a period of one hour when the animal was injected intraperitoneally with 1 c.c. of the same sample of milk. The pig died during that night.

Control: A nonsensitized pig was injected intrathecally with a few drops of the same sample of milk. A very transitory irritability was noticed. No hopping movement, paralysis of the hind legs or dyspnea. Normal inside of one minute.

Some minutes later the guinea-pig was injected intraperitoneally with 1 c.c. of the same milk. Still alive and well at the end of three days.

After these experiments were conducted, the baby was tested with the following foods that the mother had been eating as a regular part of her diet; lactalbumin, banana, beef, cocoa, corn, spinach, egg white, egg yolk, casein, wheat, potato, peach, salmon, orange, veal, carrots, rice, plum, oats, pork and peas.

Definitely positive reactions were obtained in the cases of wheat, beef, yolk, salmon, veal and carrots. Oats and peas were questionable.

DISCUSSION

A discussion of the experiments reported is hardly necessary any more than to call attention to the various points each experiment brings out. That there is nothing in the breast milk of a woman when she is not eating eggs, which is capable of reacting anaphylactically with egg protein, is shown in Experiments 1 and 6. In experiment 1 the breast milk was used as a sensitizing dose and in Experiment 6 it was used as a toxic dose. This was advisable because, as has been stated, if such protein substance were present in small quantity in the milk its sensitizing action might be masked by the relatively large amount of normal milk proteins present. Experiment 6 rules out this possibility.

Experiment 2 is very suggestive that after eating eggs, egg protein was present in the breast milk although it was not conclusive. Probably the reason why so slight a reaction was obtained in this case is because of the small amount of egg and large amount of milk protein present in the sensitizing injection.

Experiments 3, 4, 5 and 7 are proof that egg protein was present in the breast milk of three different women after they had taken eggs in their diet.

While it is not within the intended scope of this paper to discuss the clinical possibilities opened up by the work herein reported, a few points deserve mention at this time.

First, the mother in Case 2 was eating moderately of eggs, only one daily, and yet egg protein was present in demonstrable quantity in the breast milk. That the egg was causative in the disturbance of the child is shown by the subsequent history of the case. The mother was taken off eggs as a result of the experiment which has been reported. Two days later, the baby began to cry less and to sleep all night. The stools became of normal color and in a few days had lost their curdy nature almost entirely. One week later the infant was having one or two stools daily; there was almost no curd; they were normal in consistency and color; the child was sleeping all night and

crying very little during the day time, and the rash had disappeared from the face and forehead. This would have been classed as a case of over-feeding. When the infant did not improve on the reduction of food to the normal quantity for a child of its weight, we would have said the trouble was too much fat. Yet, when the offending protein was removed from the diet of the mother, the condition of the child cleared up without any reduction in the fat.

Only the protein of one food has been demonstrated to have come through the breast milk in these cases. However, it is very probable that other types do as well. This point is being investigated at the present time. The multiple sensitization of two of the infants in this series would suggest this very strongly since it is not probable that multiple sensitization would have been inherited when the mothers were not conscious of any ill effects from eating these foods. Furthermore, O'Keefe has shown that not one of the mothers in his series of ten cases gave positive tests to any of the proteins to which the infants were susceptible. This has also been true in several of my own cases not included in this series. It seems, then, that sensitization of the infants must have been actively attained and have come about only as a result of the ingestion of the foods contained in the breast milk.

A fact that has long been known, namely, that one infant will thrive on breast milk that another cannot tolerate, is easily explained on the grounds of the facts reported in this paper. Unless the second infant were also sensitized to the proteins contained in the milk we would expect nothing else.

CONCLUSIONS

1. It has been established that egg protein may be present in the breast milk after the ingestion of moderate quantity of egg at least by some nursing mothers.
2. It has been demonstrated as conclusively as possible under the conditions of the experiments that it may cause disturbance in the nursing infant under these conditions.
3. It is deemed probable that this may occur with almost any other food that the mother may eat.
4. It is contended that we must revise our ideas as regards the diet of the nursing mother and must study our cases of food disturbances in the nursing infant from the standpoint of what the mother is eating.

I am deeply indebted to Dr. W. P. Larson, Professor of Bacteriology of the University of Minnesota Medical School for many valuable suggestions throughout this work.