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Research Article

PASSAGE OF NATIVE PROTEINS THROUGH THE NORMAL GASTRO-INTESTINAL WALL[1]

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The view most generally held to-day by the physiologist, chemist and clinician is that undigested antigens are not absorbed through the normal gastro-intestinal wall; and that when such absorption does occur, it is because abnormal or pathological conditions exist, such as stasis, the excessive flooding of the intestines with protein foods, lessened activity of the digestive enzymes, altered conditions of the intestinal mucosa, and greater permeability of the intestinal wall characteristic of new-borns and sucklings. There is some evidence for the absorption of unsplit proteins under normal conditions but this is regarded as a fortuitous occurrence. In the light of the conflicting opinions present in the literature and the important rôle this subject plays in allergy and other conditions as yet not clearly defined, we determined to reopen the problem.

PART I. ANIMAL EXPERIMENTS

Methods

Guinea pigs were obtained from a reliable source. They were observed for a week and we used only those which remained healthy and gained in weight. Both young and mature animals were included in order to determine the influence of age. A large number (493) were studied in order to arrive at some approximation of the incidence of this phenomenon under the conditions to be described.

We used the anaphylaxis test as the biological method of choice to determine whether native antigens had entered the blood stream from the gastro-intestinal tract. As we have previously shown (1), the Schultz-Dale uterine strip method is less reliable as an index of a state of hypersensitiveness. The difficulties in detecting foreign protein in the blood by precipitin methods are considerable, not only because the material is so greatly diluted, but, as has been frequently shown, because it leaves the blood stream so rapidly.

It is essential to use a protein that is foreign to the diet for the reason that animals develop an immunity to foods they eat regularly (2). Such substances as crepitine, originally used by Richet (3) in his experiments on anaphylaxis, or

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vegetable proteins such as castor bean or cottonseed, which contain primary toxic substances, were not considered. The protein foods that offered the greatest possibilities for use in our experiments were milk and egg. Egg white was excluded from further consideration because it has been shown to be extremely resistant to digestion. Cow's milk, which is not toxic and is well tolerated by the guinea pig, was used; innumerable experiments already recorded in the literature and large numbers of negative reactions reported in our protocols offer evidence for the absence of any toxic properties in milk when given to the guinea pig intravenously. Fat may tend either to delay the passage of food through the pylorus or to produce diarrhea; we therefore skimmed all cream from the milk. A few control experiments carried out with horse serum are included in the protocols below.

In one series of experiments animals were sensitized orally and shocked by intravenous injection; in another they were sensitized by intraperitoneal injection and shocked orally; and in a final series they were sensitized and shocked orally. Before each feeding experiment the animals were placed in a clean metal cage and observed for 3 to 5 hours, during which period they were not given food. For the oral administration the animals were held gently and the milk was fed by mouth from a glass syringe with a metal tip. Any liquid which dripped out of the mouth was collected in a dish and refed, so that the total amounts ingested were known. This procedure took an inordinate amount of time but we believed it essential to avoid forced feedings.

Measurements were made of the anatomical capacity of the guinea pig stomach and we learned that the stomach of an animal weighing between 250 and 350 grams could hold 15 cc. with ease. Individual feedings were limited to that amount. This was to obviate any criticism relative to excessive flooding of the gastro-intestinal tract.

The animals were kept under normal conditions and observed for 3 to 4 weeks before being retested. Only animals that remained healthy and gained normally during this period were used for the final tests recorded in the protocols.

The respiratory tract serves as an admirable portal of entry (4). Animals which coughed were discarded in order that false results, due to the aspiration of antigen into the respiratory tract, might be avoided.

It must be conceded that experimental procedures can at no time be regarded as being absolutely normal, but certainly tying off loops of the intestinal tract, the use of stomach tubes, the production of stasis, flooding of the intestines and prolonged starvation of the animal constitute distinctly abnormal conditions. Such conditions were strictly avoided in our experiments. We believe that the physical state of the animal, the protein food used, and the care observed in handling and feeding warrant the assumption that our experiments were carried out under generally normal conditions. Particular stress is laid on the fact that the intestinal tract was not traumatized and that the protein food, although foreign to the natural diet of the guinea pig, is non-irritating, non-toxic and well tolerated by the animals.

PROTOCOLS

A. Sensitization of young animals

1. *By feeding repeated doses of milk:* There were 326 animals in this group, 250 to 350 grams in weight, each fed a total of 80 cc. milk. After

an incubation period of 3 weeks an injection of 1 cc. of raw skimmed milk was given intravenously.

One hundred and sixty-one showed symptoms of anaphylactic shock and 165 did not, so that approximately 50 per cent of the animals were sensitized orally. Of the positive animals 51 died in anaphylactic shock (++++), 80 showed marked symptoms with recovery (+++ and ++) and 30 showed a moderate degree of anaphylaxis with recovery (+).

2. *By a single feeding of milk:* We attempted to learn whether large amounts of antigen are needed to sensitize, or whether small amounts may gain entrance through the normal alimentary wall. There were 22 animals, each given a single feeding of 5 cc. milk. When given an intravenous injection of 1 cc. milk 3 weeks later, 6 showed symptoms of anaphylaxis, 2 of which died in shock and 4 showed symptoms with recovery; 16 were negative.

B. Sensitization of older animals

Can antigens traverse the intestinal wall in older animals as they do in a large percentage of younger animals? In this lot were 47 animals ranging in weight from 600 to 1000 grams.

1. *By feeding milk:* Of this group 19 were fed, as described above, but with somewhat larger doses of milk. On primary injection with 1 cc. milk 8 manifested positive symptoms of anaphylaxis and 11 were negative. Of the 8 positive cases, 3 died in shock and 5 showed definite symptoms of anaphylaxis with recovery.

2. *By feeding horse serum:* In a similar manner 10 mature animals were fed horse serum. After the injection of 1 cc. of horse serum 3 weeks later 1 died in shock, 2 manifested symptoms with recovery, and 7 were negative.

3. *Sensitization of pregnant animals:* There were 18 animals which were fed during the latter part of pregnancy and injected after parturition. Of these 6 were positive, 1 of which died in shock, 5 showed symptoms and recovered, and 12 were negative.

C. Sensitization of new-born through feeding

Many attempts were made to feed animals a few days old, but the mortality was so great that this phase of our work was given up as unfruitful.

Horse serum was used in a few litters. Young animals seem to tolerate this better than milk. We were successful in feeding 1 litter of 4 animals a week old. Proportionately smaller doses were used (see Table I). Of these, 2 died in shock and 1 showed symptoms with recovery following the intravenous shocking injection after an interval of 3 weeks.

TABLE I

Summary of feeding experiments

Number of animals	Type of * animal	Sensitization *		Shock *		Result *	
		Feeding	Ip. inject.	Iv. Inject.	Feeding	Positive	Negative
326	Young	80 cc. M.		1 cc. M.		161	165
19	Old	150 cc. M.		1 cc. M.		8	11
10	Old	60 cc. HS		1 cc. HS		3	7
18	Pregnant	200 cc. HS		1 cc. HS		6	12
4	New-born	20 cc. HS		0.5 cc. HS		3	1
22	Young	5 cc. M.		1 cc. M.		6	16
44	Young	80 cc. M.			5 cc. M.	14	30
8	Old	150 cc. M.			5 cc. M.	1	7
42	Young		5 cc. M.		5 cc. M.	11	31

* Young, 250 to 350 grams; old, 600 to 1000 grams; pregnant, during latter part of pregnancy; new-born, 65 to 75 grams (1 week old); ip., intraperitoneal injection; iv., intravenous injection; positive, definite anaphylaxis with recovery or lethal anaphylaxis; negative, no symptoms or suggestive anaphylaxis; M, skimmed certified milk; HS, normal horse serum; 5 cc. M., 1 small feeding; 80 cc. to 200 cc., several feedings of 15 cc. each.

D. Sensitization by injection; shock by feeding

In this series an attempt was made to learn how rapidly ingested antigens may enter the systemic circulation.

Young animals (250 to 350 grams) were used. They were all sensitized by intraperitoneal injections of 5 cc. of milk, and 3 weeks later were given a feeding of 5 cc. of milk. Animals were observed for about 1 hour after feeding. Each animal was subsequently given an intravenous injection of 1 cc. of milk to determine the degree of hypersensitivity.

There were 42 animals. Oral administration resulted in 11 definite anaphylactic responses, 13 doubtful reactions and 18 negative reactions. Of the group of 11 animals, 1 died in shock, 5 manifested a ++++ reaction, 3 a ++ reaction and 2 a + reaction. The reactions occurred from 5 to 30 minutes after the feeding. The subsequent intravenous injection, in the majority of instances, demonstrated that the shock reactions from feeding were generally mild, for 10 animals showing no symptoms from feeding milk died after intravenous injection; nine of the animals which showed doubtful symptoms gave lethal or violent reactions after intravenous injection. However, in 3 instances the shock was quite as severe after

feeding as after subsequent injection. One animal, indeed, died 3 minutes after feeding.

E. Sensitization by feeding; shock by feeding

1. *Young animals:* In this series there were 44 young animals of which 3 gave a violent reaction, 11 a moderate reaction, 9 a doubtful reaction and 21 a negative response to shock feeding. In all instances the subsequent intravenous injection of 1 cc. of milk resulted in more intense symptoms of anaphylaxis, indicating that while antigens orally administered may gain entrance into the systemic circulation, in general they do not enter in as large amounts nor as quickly as when given by the intravenous route.

2. *Older animals:* A group of 8 older animals was sensitized and an attempt made to shock them by feeding. Of these 1 manifested definite anaphylactic shock with recovery, 2 were doubtful, and 5 were negative. All reacted positively after intravenous injection.

These experiments are summarized in Table I. The large number of animals that failed to give symptoms after the intravenous injection of milk serve as controls with respect to the possible toxicity of the milk.

PART II. HUMAN DATA

1. *Passage of antigen not common to the diet:* The last 3 years during the course of this study, medical students were given the Prausnitz-Küstner (5) test. They were injected with the serum of an asthmatic child sensitive to cottonseed and 24 hours later were given a teaspoonful of dried cottonseed by mouth. These students, in the majority of instances, gave a pronounced reaction at the site of the injection of serum, between 15 and 30 minutes after ingestion.

2. *Passage of antigens common to the diet:* The same method was employed in non-allergic children free from gastro-enteric disturbance. These children ranged in age from 3 to 12 years. Eighty per cent of the 20 children tested showed positive reactions to foods common to their diet such as egg, milk and nuts. We might cite one interesting example. The serum of an allergic child highly sensitive to lactalbumin was injected into the skin of a 12 year old child, perfectly well and about to be discharged from the hospital. She abstained from milk throughout the following morning. Within 20 minutes after being given a glassful of milk to drink, she developed a positive local skin reaction, demonstrating the passage of this common food antigen into the blood stream.

Acute attacks of asthma were repeatedly induced in a 9 year old child, sensitive to lactalbumin, by the ingestion of fresh cow's milk in amounts not exceeding several teaspoonfuls. His serum contained specific antibodies to lactalbumin which we succeeded in transferring by the Prausnitz-Küstner method. Guinea pigs were also passively sensitized with the serum. On one occasion, when this patient was tested intracutaneously

with lactalbumin, he developed immediate symptoms of anaphylactic shock from which he recovered.

It is clear from these observations that unsplit antigens, both foreign and common to the diet, can traverse the intestinal wall of normal adults and children. In a highly sensitive allergic individual a small amount of antigen taken by mouth may produce shock.

PART III. HISTORICAL CRITIQUE

The literature on this subject is most extensive and to present it in its entirety in condensed form would serve only to confuse the reader; we shall therefore only briefly summarize those phases which have the most direct bearing.

Lower animal

In the oldest experiments dealing with this question, the passage of proteins through the intestinal wall was thought to have been demonstrated by finding albumin in the urine after large amounts of protein were fed, particularly egg white (6, 7).

Experiments with loops of intestine were introduced as far back as 1869. Portions of the intestinal tract were exposed and tied off, protein substances injected into the lumen of these loops, and after various intervals the amount of nitrogenous material that remained in the loop was determined. Some (8, 9, 10) who found that a difference existed between the total amount of protein injected and the total nitrogen which remained interpreted this as indicating that undigested protein had passed through the wall of the loop. Such a difference however was not found by all investigators (11, 12). The outstanding experiments performed by this method were done by Mendel and Rockwood (13) who used edestin as the protein substance. The edestin remaining in the loop was recovered, chemically identified, and measured. A reduction was shown. The authors believed that the diminution was due to a passage of some of the edestin in its native state through the wall of the loop.

Mills et al. (14) and Hektoen et al. (15) went further. They fed animals tissue fibrinogen and thyroglobulin respectively, and were able to show the presence of these proteins in the blood stream. Digestion of both these complex protein substances destroys their physiological properties so that their detection in the blood is evidence for their passage in an unchanged state.

However, it was only after immunological methods were introduced that protein specificity could be demonstrated. By the use of specific antisera it was shown that unchanged ingested antigens were present in the blood and urine (16, 17, 18, 19, 20, 21, 22) and conversely, by testing against the ingested antigen, specific antibodies were found in the blood and urine (23, 24, 25, 26, 27). Hamburger is representative of a group of investigators (28, 29, 30) who, though using the precipitin method, apparently found it difficult to demonstrate the presence of antigen in the blood stream after ingestion, and voiced the belief that it occurred only under pathological conditions and particularly in the newborn.

The most convincing evidence for the passage of antigens through the intestinal wall has been brought forth by the use of the anaphylactic method. Animals were directly sensitized and shocked by feeding antigens (2, 3, 22, 26, 27, 31, 32, 33, 34, 35, 36, 37, 38, 39). The presence of antigen or antibodies in the blood or urine was also determined by indirect anaphylactic tests, i.e., normal

animals were passively sensitized by the blood or urine of animals sensitized through ingestion (37, 40, 41).

It was also shown (42, 43) that the fetus could be actively or passively sensitized to foods ingested by the pregnant female.

Many investigators, as Arloing et al. (44) and Makaroff (45) who followed the teaching of Besredka (46) that proteins did not pass unchanged through the intestinal wall, believed, on the other hand, that if bile or other irritants were fed to the animal before the proteins, the irritation rendered the intestinal mucosa more permeable and permitted the passage of unchanged proteins.

Human data

Czerny and Latschenberger (47) worked with a human being who had a sigmoid fistula. They introduced various protein substances into the fistula and found that there was a diminution in the nitrogen content after a lapse of time. This observation is similar to the results obtained with the intestinal loop experiments performed on animals.

Alimentary albuminuria may occur in the normal individual and is due to the excretion of undigested protein and not to the excretion of endogenous protein resulting from a pathological lesion of the kidney. This has been demonstrated by specific precipitin tests with the excreted protein, and by actively sensitizing normal guinea pigs with the foreign protein present in the urine (7, 16, 21, 26, 48, 49, 50, 51, 52, 53).

The presence of ingested food proteins in the blood stream of normal individuals has been shown by precipitin and indirect anaphylaxis tests (17, 19, 53, 54, 55, 56). Specific antibodies to food proteins found in the blood of allergic individuals have given precipitates with specific antigens, and have passively sensitized normal guinea pigs (41, 57, 58, 59).

Normal individuals, injected intracutaneously with the serum of patients sensitive to some food, have given positive local reactions (Prausnitz-Küstner test) when food to which the patient was sensitive was eaten (60, 61, 62, 63, 64).

Tissue fibrinogen ingested by a group of investigators (14) markedly influenced the blood coagulation time. This complex protein substance must have entered the blood stream unchanged for, as we have already stated, digestion destroys its physiological action.

Innumerable cases of food intolerance have been observed in which positive protein skin tests were obtained, showing that a particular food ingested was the direct cause of the allergic disturbance. This work has been summarized by Rowe (65) and Laroche et al. (66).

Anaphylactic shock and even death are recorded after the ingestion of protein foods (67, 68, 69, 70, 71, 72, 73, 74). Bouteil (75) reported an interesting case of an adult who received 3 rectal injections of horse serum at monthly intervals. After the third injection this patient went into anaphylactic shock.

Ratner (76) has shown that the human fetus can be sensitized in utero as a result of the excessive indulgence in protein foods by non-allergic women during pregnancy. Allergic mothers sensitive to foods may in the same manner transfer specific antibodies to the fetus (77). Thus, the fetus can be actively or passively sensitized to foods in utero and when the infant or child ingests these specific foods for the first time it may manifest allergic reactions.

Certain authors (54, 48, 71, 73, 78, 79) have observed that children suffering from gastro-intestinal disturbances can become sensitized to foods which they tolerated prior to their illness.

It has been reported (80, 81, 82) that individuals who ate horse meat were sensitized to horse serum and manifested anaphylactic symptoms and in some instances have died when given primary injections of horse serum. This was shown to be due to the presence of horse meat antibodies in the blood of these individuals, the result of hippophagy.

PART IV. DISCUSSION

We believe that the historical survey gives sufficient evidence to warrant the conclusion that unsplit antigens enter the blood stream directly from the gastro-intestinal tract. However, we find that little consideration has been given to the important question as to whether this passage occurs through the normal intestinal wall and in the course of normal digestion.

Because they found it difficult to show this phenomenon under normal circumstances with the methods they employed, many investigators introduced artificial and pathological conditions to demonstrate it; and they therefore postulated that only when the permeability of the intestine is increased as a result of intestinal stasis, deficient enzyme action, or damage to the intestinal mucosa, was it possible for unchanged proteins to pass through the intestinal wall. Among other causes accounting for the difficulty in demonstrating the presence of unchanged food antigens and specific food antibodies in the blood stream and urine of normal animals are the following. First must be mentioned the fact that small numbers of animals were used by many of the investigators. Secondly, inadequate amounts of protein have been fed to animals and human beings who subjected themselves to experimentation. Thirdly, the difficulties of detecting foreign proteins and antibodies by the precipitin method are considerable not only because the material is so greatly diluted in the blood and urine, but, as has been frequently shown, ingested foreign proteins absorbed through the intestinal tract leave the blood stream as a rule within a few hours, and disappear from the urine within 24 hours. The attempts made to sensitize animals actively or passively with the blood or urine containing foreign protein or specific antibodies have also met with many failures largely because of the last-named factors.

Pathological lesions and other factors which increase the permeability of the intestinal wall undoubtedly enhance the absorption of proteins, but it is our contention that under normal conditions the absorption of proteins occurs with greater regularity than is generally believed.

As we have already pointed out experimental procedures can at no time be regarded as being carried out under absolutely normal conditions. Our experimental conditions were normal in so far as the gastro-intestinal tract was not traumatized, and in so far as we used no chemical irritants. We chose the direct anaphylaxis method as that subject to fewest criticisms. Cow's milk, while it is not natural to the diet of the guinea pig, contains no primary toxic substance. Our animals were observed for three weeks after the original oral administration of the foreign protein and during this period

they showed no evidence of diarrhea and no loss in weight; at the end of this time, before they were retested, they appeared healthy and active. We assumed therefore that the experiments were carried out under generally normal conditions.

Our experiments on a large number of animals show that at least 50 per cent can be sensitized through natural ingestion and that the passage of these antigens takes place in the adult as well as in the newly-born animal. Antigens, when fed, generally enter the circulation in small amounts. Though a single small dose may occasionally sensitize, moderately large amounts are necessary to sensitize animals with any degree of regularity. At times, also, antigens are absorbed in sensitized animals in large enough amounts to produce shock reactions which are as profound as those observed after intravenous injection.

In the human subject our observations show that cottonseed as well as foods common to the diet, such as milk, when taken into the stomach may enter the circulation in demonstrable amounts. This occurs not only with allergic patients but also with normal individuals and in the adult as well as in the young.

Schloss and his co-workers (56), using immunological methods (precipitin and indirect anaphylaxis tests), showed that ingested native proteins (milk, egg, almond) enter the blood in normal young children. Walzer et al. (62), who applied the Prausnitz-Küstner test, found that 88 per cent of normal adults whom they studied showed the presence of proteins of egg and fish in the blood after ingestion. Walzer particularly stresses the physiological nature of this passage and, because of its frequent occurrence in the average individual, believes it to be a normal phenomenon.

Proteins may enter the blood stream from any part of the bowel even as low down as the rectum. The entrance of these antigens into the circulation apparently takes place below the stomach for it is well established (83) that there is little absorption of any kind from the gastric mucous membrane. It is doubtful whether any chemical stimulus of the gastric mucosa is needed to open the pylorus for it relaxes at intervals and if the gastric contents are under any pressure the liquid part is squirted out while the more solid parts are retained. The intestinal contents are propelled by large rapid waves, which run down the bowel from time to time, called "peristaltic rush" by Meltzer and Auer (84). It is conceivable therefore that antigens in fluid form can pass the pylorus and may enter the small and large intestines in an exceedingly short time. This would explain the death that occurred in one of our animals within 3 minutes after ingestion of the foreign protein; the rapid passage of cottonseed and milk in the human subjects after ingestion, and the observations of Mills et al. (14) that ingested fibrinogen entered the circulation within 2½ minutes.

Another factor which may assist in the absorption of unsplit proteins is to be found in the fact that all proteins are not digested with equal

avidity. Many vegetable proteins escape digestion. It has been shown by Mendel and Lewis (85) in the human being, and Bateman (86) in the animal, that raw egg white largely escapes digestion. It has also been demonstrated that the proteins of raw milk are not readily digested and therefore the soluble whey proteins might easily be absorbed.

Thus, the inaccessibility of proteins to enzyme action and their rapid passage through the intestinal tract might allow for the ready entrance of undigested proteins into the blood stream. The process must be in the nature of a purely mechanical filtration because of the rapidity with which the proteins enter the blood stream.

If antigens enter the circulation regularly, one must attempt to explain why all individuals are not sensitized to these foreign proteins. Quantity and time are largely essential in the mechanism of sensitization. If antigens enter in small amounts and at frequent intervals, it has been shown by Wells (2), Schloss and his co-workers (56), and Laroche et al. (66) that an immunity is established. On the other hand, if antigens enter in particularly large amounts and at infrequent intervals, then the animal or man may become profoundly sensitized and the subsequent ingestion of even small amounts of antigen may produce allergic manifestations, shock, and even death.

It is feasible to speculate that throughout the ages animal and man must have developed defense mechanisms, for otherwise they would have succumbed to the ingestion of new and strange protein foods. The first line of defense is the hydrolysis of the ingested proteins by the digestive enzymes; next, the general impermeability of the intestines to proteins in a native state. The second line of defense after the antigen has entered the blood stream consists in the development of specific antibodies which may divert the absorbed protein from the sensitized cells, thereby rendering it harmless; and next, the important function of urinary excretion which tends to rid the circulation of foreign proteins. A third line of defense which does not pertain to body processes is the development of various methods of cooking and preparing foods which render proteins more digestible; and lastly, the general tendency of man and animal to indulge in new foods sparingly and to adhere to a more or less limited diet.

We believe that although abnormal conditions, which tend to increase intestinal permeability facilitate the passage of proteins through the intestinal wall, absorption of protein occurs under normal conditions with greater regularity than is generally held. This normal absorption may conceivably serve the useful purpose of constantly maintaining the human body in a state of immunization against habitual protein diets.

CONCLUSIONS

Mature, as well as young animals can be sensitized and shocked by the oral administration of protein foods. The incidence in a large series of

animals was shown to be as high as 50 per cent. The experiments were carried out within physiological limits. We believe that these experiments offer conclusive evidence for the fact that unsplit proteins pass the intact intestinal wall under normal conditions.

In the human being it is shown that proteins, natural as well as foreign to the diet, pass the intestinal wall and may enter the blood stream under physiological conditions at all ages.

The body is provided with certain defensive measures which impede the entrance of proteins in an unchanged state into the blood stream and tissues. These include the general impermeability of the intestines, the digestive enzymes, specific antibodies and the excretory function of the kidneys. Denaturation of proteins by culinary processes also aids the body in this defense. When the defense mechanisms fail to act and native antigens enter the blood stream in large enough amounts, man or animal may become profoundly sensitized and the ingestion of small amounts of antigen thereafter will produce allergic manifestations and may even result in death.

Although abnormal or pathological conditions of the intestinal mucosa increase intestinal permeability and facilitate the passage of unchanged proteins, we believe that under normal conditions absorption of unaltered protein occurs with great regularity. This normal absorption may conceivably serve the useful purpose of constantly maintaining the body in a state of immunization against habitual protein diets.

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