APPENDIX B

STUDIES ON SUPERSENSITIVITY*

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Among the idiosyncrasies to foodstuffs the supersensitivity to fish has so far received little attention. Since one of us (K.) suffers from this affection, we took this opportunity of investigating the mechanism of the reaction and the serological conditions concerned. In the course of the work a series of controlled tests were carried out on the other one (P.), who is a hay-fever patient, and on several non-sensitive colleagues who kindly placed themselves at our disposal. The patient, an otherwise healthy man of 24, is known to have been fish-super-sensitive since the age of 6. After eating the merest trace of marine or freshwater fish the following symptoms appear:

After half an hour itching of the scalp, neck, lower abdomen, dry sensation in the throat; soon afterwards swelling and congestion of the conjunctivae, severe congestion and secretion of the respiratory mucous membranes, intense fits of sneezing, irritating cough, hoarseness merging into aphonia, and marked inspiratory dyspnœa. The skin of the entire body, especially the face, becomes highly hyperaemic, and all over the skin of the body there appear numerous very itching wheals, 1-2 cm large, which show a marked tendency to confluence. Increased perspiration has not been noted. After about 2 hours heavy salivation starts and is followed by vomiting, after which the symptoms very gradually fade away. Temperature, cardiac and renal function have always been normal. After 10 or 12 hours all the symptoms have disappeared; only a feeling of debility persists for a day or so. After each attack there is a period of oliguria and constipation; this may be due to dehydration and vomiting, but perhaps it is better explained by retention of water similar to what occurs in serum sickness.

What may perhaps be regarded as an abortive form of the illness is the patient's observation that after careless working with fish-glue he has occasionally had urticaria of the lips. The symptoms and signs of fish supersensitivity can have no psychological basis. They have frequently occurred when he had no idea of having eaten any fish e.g. once after eating tunny fish which he had taken for meat; another time after eating parsley which had been chopped up on a board previously used for cutting up anchovies. But the patient has found that he can cat caviar with impunity.

*Centralbl. F. Bakteriol. 1. Abt. Orig. 86, 160-9(1921). Translated from the German original by Carl Prausnitz
The active substance appears to occur practically only in the muscle flesh of bony fish. It occupies a peculiar position among the agents of supersensitivity, since for our patient fish is completely harmless in the raw state and only becomes poisonous when heated (cooked, baked or fried).

Starting from these observations it was first tried to produce the signs of supersensitivity by parenteral administration of the fish antigen. A standard solution was prepared by mincing fresh marine fish (usually haddock), boiling it in ten times its weight of distilled water, filtering through paper and sterilizing the filtrate for half an hour in the steam sterilizer. The clear, non-opalescent fluid thus obtained proved inactive on the patient's conjunctiva, but highly active on intradermal injection. After intradermal injection of 0.1 c.c. of the standard solution (the utmost care being taken to avoid subcutaneous injection) there developed at the site of injection within 10 minutes a very itching weal which rapidly, under our eyes, grew to about 4 cm in diameter. The fully developed weal was raised high above the surrounding skin, white, with an indented margin; it was surrounded by a deep red flare about 10 cm wide. After 20 minutes there developed the syndrome of severe generalized intoxication previously described (urticaria of the entire body, intense congestion of the conjunctivae and upper air passages, irritating cough, dyspnoea). The generalized signs, which we were able to produce repeatedly in the same form, gradually fade away after several hours. Subcutaneous injection of 1 mg atropine sulphate quickly and completely relieves the respiratory symptoms, 0.1 mg 'suprarenin' the urticaria. But even after a day, sometimes even 2 days, oedematous infiltration of the injection site persists. In our experience this oedema is very characteristic of positive supersensitivity reactions.

A distinct local reaction could be obtained by intradermal injection of as little as 0.1 c.c. of a thousandfold dilution of the standard solution, but not by 0.1 c.c. of a ten-thousandfold dilution. The limiting active dose therefore corresponds to the boiled extract of about 0.01 mg of fish muscle.

However, a 50 per cent watery solution obtained by leaching fish muscle in the cold was completely inactive in the patient. If this solution was boiled, a precipitate formed which was inactive, whilst the filtrate was weakly active (about 1000 times less than the original standard solution described previously). After heating to 50ºC the solution remains inactive. At 55ºC—i.e. the lower limit of protein coagulation—a toxic effect begins to appear; above 60ºC its toxicity is found not to differ from that of the boiled extract. In agreement with this finding is the fact which greatly astonished our patient, that he can eat raw fish (5 g!) without the slightest discomfort. It appears, therefore, that the active agent is only formed in fish muscle when it has been heated. Evidently in the fish extract prepared in the cold and filtered, small particles of finely dispersed fish protein had remained in suspension and from them antigen was formed on boiling.

The intradermal tests have proved that even after technically unobjectionable injection of sufficient amounts of antigen* so great an absorption of the poison can take place so rapidly that the picture of a severe general intoxication will develop. This observation is not unique.

With hay fever similar severe reactions have been described by Dunbar, Prausnitz and others after subcutaneous injection of the active pollen protein. But according to our

* 1000 times the quantity required to produce a local reaction
investigations the same severe syndrome can be produced even by the intradermal injection of pollen protein. The other one of us, a hay-fever patient, was given 0.1 c.c. of 1 per cent solution of rye pollen protein intradermally§ that is 2000 times the minimum amount required to produce a conjunctival reaction in this patient. After 10 minutes there developed a white, raised, very itching wheal with indented border, 22 x 17 mm in size, with a deep red flare 60 x 45 mm. Ten minutes later intense conjunctival congestion with severe lachrymation, a burning, itching sensation and chemosis. After a further 10 minutes there was severe inspiratory dyspnoea, panting respiration, stridor, distressing cough. The wheal was now 45 x 23 mm, the flare 200 x 90 mm in size. The eyelids became very oedematous, the palpebral fissure narrowed to a slit, the face so swollen as to be almost unrecognizable. This disfigurement only gradually disappeared after several days. For several days the site of injection presented a doughy swelling and remained painful.

II

In order to determine the active principle the original standard fish solution was first mixed with an eightfold volume of absolute alcohol, and after standing for 24 hours was filtered through paper. The filtrate was evaporated to dryness on the water-bath; from 10 cc of the standard solution 0.027 g of a greenish-yellow amorphous material was obtained. It was suspended in 10 cc of normal saline solution; it proved quite inactive on intradermal injection. The alcohol precipitate (0.035 g from 10 cc of standard solution) was also suspended in 10 cc normal saline; this solution, just like the original standard solution, was active in one thousandfold dilution. Therefore the antigen had passed quantitatively into the alcohol precipitate. The minimal active dose of the alcohol precipitated antigen is 35 x 10^-5 mg.

No active substance can be extracted from fish muscle by alcohol or ether in the cold or by heat.

The substance was then tested for dialysability. Ten cc of the standard solution were dialysed in a Schleicher and Schüll dialysing sheath for 24 hours against frequent changes of distilled water. The dialysate (4 litres) was inspissated on the water-bath to a volume of 20 cc; it proved inactive by intradermal test. The fluid within the sheath had retained its original activity. It gave a distinct biuret and Million reaction.

Therefore the antigen is neither fat nor lipid. It is allied to the proteins but does occupy a peculiar distinct position: coctostable antigens, it is true, have long been known, e.g. pollen protein. But what is new is the fact that the active substance is only formed when the solution is heated beyond the temperature of denaturation of protein. We would assume that it still is a fairly high molecular decomposition product of fish protein, since it is precipitated by alcohol, is non-dialysable and gives the above-mentioned protein reactions.

If the standard solution is treated in the cold with 10 per cent acetic or 10 per cent nitric acid, a precipitate forms; this, like the filtrate after neutralization, is intradermally inactive. If acetic acid is added to the standard solution to a concentration of 0.1 per cent in the cold, its activity is reduced to about one-tenth; when boiled this solution becomes quite inactive. After treatment with 3 per cent potassium hydrate the standard solution remains clear and active.

It was not possible to detoxicate the standard solution with mildly acidified pepsin of proved activity, nor with alkaline trypsin.

§We are indebted to Professor Dunbar for kindly supplying this valuable preparation
The fact that the patient can eat caviar without suffering any trouble suggested testing the various organs of fish for activity. Only the cross-striated muscle of bony fishes was found to contain the antigen in considerable quantity. Decoctions of stomach and liver were very weakly active (about 1000 times less so than the standard solution described above). Inactive were decoctions of spleen and roe, as well as pure, undiluted fish serum, both raw and boiled. Of cartilaginous fishes the ray proved to be about ten times weaker than the bony fishes investigated. Naturally whale flesh was inactive.

III SEROLOGICAL INVESTIGATIONS

In view of the strictly specific character of the reaction described the serum of the supersensitive individual was tested for the presence of antibodies. With the most varied testing arrangements it was impossible to demonstrate in vitro precipitins or complement-binding substances in his serum or in that of normal persons.

Following the observation of Bauer (Miinch. med. Wochenschr 1911, p. 71) that in serum sickness the patient's serum agglutinates the corresponding erythrocytes, we tested our patient's serum for activity against fish red blood corpuscles, but with no result. Nor could we discover in a test on the human being any neutralizing substances in the serum: mixtures of equal parts of the standard fish solution and freshly drawn serum of the patient, as well as of a normal person, were kept for 2 hours at 37°C and overnight in the icebox; their toxicity by intradermal injection to our patient remained uninfluenced.

These results show a close agreement with the similar behaviour of pollen protein in the hay-fever patient. This substance is also a coctostable protein-like substance which, it is true, behaves differently towards acids and alkalis but is also very resistant to digestive enzymes. Both substances are strictly specific for the corresponding supersensitive persons, although they show a considerable quantitative difference in their active dose. Their chief difference is that the poisonous pollen protein is preformed, whilst the poisonous substance from fish does not develop until the protein has been denatured. Pollen protein, injected subcutaneously or intradermally to the hay-fever patient, produces exactly the same symptoms as fish extract in the fish-sensitive individual. A further difference, though hardly so important a one, is the fact that pollen protein is inactive after oral, fish extract after conjunctival administration.

The reactions observed show a remarkable resemblance to those of genuine anaphylaxis. True, there is one difference: in fish- and pollen-supersensitive persons after intradermal injection the reaction sets in almost without any incubation period—within a few minutes—and fades relatively soon; in the person sensitive to serum or tuberculin the intradermal reaction only begins after several hours and remains at its height for days. Still, these differences might only be of a quantitative nature. Whether the forms of supersensitivity discussed here come under the heading of true anaphylaxis would most readily be decided if they could be passively transferred to non-susceptible beings. In guinea-pigs it was impossible to produce passive anaphylaxis by intra-peritoneal injection (1 or 2 cc) of the fish-supersensitive patient's serum followed 24 hours later by intravenous injection of 0.5 cc standard fish solution. By a similar technique, using the serum of a hay-fever patient and pollen protein, again no result was obtained.
The attempt was therefore made to transfer the supersensitive state passively to non-susceptible human beings. The technique used in guinea-pigs was out of the question, since corresponding to the weight of the individual to be tested far too much serum would have to be taken from the donor, and above all because of the danger of producing a severe-anaphylactic shock. We therefore tried to localize the anaphylactic reaction within the skin by injecting both reactants (serum and antigen) intradermally into the same spot of skin.

1. Mixtures of standard fish solution with the fish-sensitive patient's serum in varying proportions, injected intradermally to normal persons in 0.1 cc amounts, were inactive.

2. Even after being kept for 2 hours at 37° C and for 24 hours in the icebox, they failed to become active.

3. A positive result was only achieved when, conforming exactly to the passive anaphylactic experiment in the guinea-pig, the serum was first injected intradermally and the standard fish solution was injected the following day into the same spot of skin.

On July 19th, 1920, a person, not sensitive to fish solution, received into the abdominal skin intradermal injections of 0.1 cc of the following substances:

- (1) Serum of the fish-sensitive patient, undiluted;
- (2) Serum of the fish-sensitive patient, diluted 1 in 10;
- (3) Serum of the fish-sensitive patient, diluted 1 in 100;
- (4) Serum of a healthy person, free from any idiosyncrasy;
- (5) Normal saline solution.

On July 20th, 1920, 0.1 cc of standard fish solution was injected into each of these spots and (6) into an untreated spot of skin. The arrangement of the wheals was as follows:

<table>
<thead>
<tr>
<th>1</th>
<th>left</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>right</td>
</tr>
<tr>
<td>5</td>
<td>navel</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

After 15 minutes a marked subjective and objective reaction was present only in the spots 1, 2 and 3 pretreated with the specific serum. It was strongest where the undiluted serum had been administered. The control wheals 4, 5 and 6 showed only a trifling traumatic reaction (see Table 1).

After 1 hour the wheal and flare in the skin spots 1, 2 and 3, pretreated with specific serum, were still about the same size as after 15 minutes, but already the border between wheal and flare had become indistinct and the flare was starting to fade. On the next day there was still in these spots the distinct oedema which was always seen in the fish-sensitive patient after intradermal injection of the fish antigen. But the spots 4, 5 and 6 remained free from any trace of oedema.

The experiment was repeated in this person, who incidentally is supersensitive to pollen, and in two others, one male and one female, who are free from any idiosyncrasy: the result was the same. In a further person, supersensitive to peptone, there were also differences in favour of the spots pretreated with the specific serum, but they were less marked.

*Note during the printing of this article. In promising experiments with the somewhat similar syndrome of acquired supersensitivity of hide-dyers to 'Ursol' (quinone di-imino polymers) H. Curschmann has recently succeeded in rendering guinea-pigs passively anaphylactic to (his substance with the patient's serum (Münch. med.Wochenschr. 1921, No. 1)
It has thus been proved by this test that, according to the technique we have elaborated, the state of supersensitivity can be transferred passively to normal persons by the serum of the supersensitive individual.

It had now become obvious to try out the technique in other forms of supersensitivity. For this purpose we chose hay-fever and sensitivity to tuberculin and to horse serum. In all of them the result of numerous tests was negative. One might still consider the possibility that, e.g. in hay-fever not the serum but certain cells were the carriers of supersensitivity; as such the cells of the subcutis and cutis were the most likely ones since the skin shows a specially high degree of pollen sensitivity. To decide this question a piece of the hay-fever patient's skin, 15 sq. cm in size was excised; the subcutis and deeper layer of the cutis were carefully scraped off and ground with glass powder in an agate mortar to a fine pulp; 6 c.c of normal saline was slowly added and stirred to make a suspension which was shaken for 15 minutes and spun for 1 minute in the hand centrifuge to sediment the coarser particles. The supernatant, a slightly opalescent, blood-stained fluid, was microscopically free from cells. It was tested in three persons according to the technique used previously for testing passive transmission of pollen sensitivity—but without any result.

Possibly there is a difference of principle between fish supersensitivity and the other idiosyncrasies. But we consider it to be more probable—particularly in view of the striking resemblance already stressed between fish and pollen super-sensitivity—that such differences are merely quantitative. This assumption is favoured by the fact that the fish-sensitive individual presents an unusually high degree of sensitivity whereas the human donors used for the hay-fever and horse-serum tests were only moderately supersensitive. Perhaps we might have succeeded in transferring pollen and horse-serum supersensitivity passively by our test, if more highly sensitive individuals had been available. However, the patient used in the tuberculin test reacts so strongly to tuberculin that in his case such an assumption can hardly be justified.

According to the experiments described above it is highly probable that fish

<table>
<thead>
<tr>
<th>Skin Spot No.</th>
<th>After 15 minutes</th>
<th>After one hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wheal</td>
<td>Flare</td>
</tr>
<tr>
<td>1</td>
<td>21 x 15 mm</td>
<td>120x70 mm</td>
</tr>
<tr>
<td></td>
<td>Tense, pale,</td>
<td>Bright red</td>
</tr>
<tr>
<td></td>
<td>raised to</td>
<td></td>
</tr>
<tr>
<td></td>
<td>plateau with</td>
<td></td>
</tr>
<tr>
<td></td>
<td>irregular</td>
<td></td>
</tr>
<tr>
<td></td>
<td>protrusions,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>very irritating</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>12 x 11 mm</td>
<td>80 x 50 mm</td>
</tr>
<tr>
<td></td>
<td>Same appearance</td>
<td>Bright red</td>
</tr>
<tr>
<td>3</td>
<td>12 x 8 mm</td>
<td>55 x 28 mm</td>
</tr>
<tr>
<td></td>
<td>Same appearance</td>
<td>Red</td>
</tr>
<tr>
<td>4</td>
<td>7x5 mm</td>
<td>60x25mm</td>
</tr>
<tr>
<td></td>
<td>Slightly raised</td>
<td>Pale</td>
</tr>
<tr>
<td></td>
<td>circular,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>quite insensitive</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7x6 mm</td>
<td>40x25 mm</td>
</tr>
<tr>
<td></td>
<td>Slightly raised</td>
<td>Pale pink</td>
</tr>
<tr>
<td></td>
<td>circular,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>quite insensitive</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>8x7 mm</td>
<td>55 x 30 mm</td>
</tr>
<tr>
<td></td>
<td>Slightly raised</td>
<td>Pale</td>
</tr>
<tr>
<td></td>
<td>circular,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>quite insensitive</td>
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</tr>
</tbody>
</table>
supersensitivity should be regarded as a true anaphylactic phenomenon.

IV

The possibility of active immunization against hay-fever is known from the experiments of Noon, Freeman, Dunbar, Eskuchen and others. One of us (P.) studied the question in detail in the summer of 1919; with some highly active rye

<table>
<thead>
<tr>
<th>Intradermal injection 0.1 c.c.</th>
<th>Left forearm, flexor aspect after repeated injections</th>
<th>Right forearm, flexor aspect no previous injections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard fish solution undiluted</td>
<td>Very weak local reaction Wheal 20 x 13 mm, Flare red, indistinct, no sharp margin</td>
<td>Very strong local, almost a general reaction Wheal 36 x 25 mm, Flare 135 x 70 mm, deep red On the extensor aspect a small, very irritating daughter wheal has developed</td>
</tr>
<tr>
<td>After 15 minutes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

pollen solutions supplied by Professor Dunbar he obtained promising results in two out of four hay-fever patients. The mechanism of this protection is not yet sufficiently understood. It has not by any means been proved that genuine immunity is involved here. Opposed to such a view would seem to be the fact that so far substances neutralizing the hay-fever poison have not been discovered in the serum of rabbits, goats and horses immunized against pollen. Perhaps, as Bessau suggests, this is a case of anti-anaphylaxis or katanaphylaxis. Nor has finality been reached as to the technique of this immunization. The dose employed should not be too small, otherwise success is usually not achieved. But if it is increased too rapidly, acute symptoms readily develop in the skin and respiratory passages; they may occasionally become very severe and require treatment with atropin and suprarenin (Noon, Freeman, Bessau, personal experiences). The possibility, however, of producing a transitory, perhaps even a permanent control of supersensitivity can be deduced from observations on the fish-sensitive patient. After a considerable number (about forty) of intradermal injections of different fish extracts had been earned out during about 6 weeks, always on the flexor aspect of the left forearm, a very marked local reduction of his sensitivity was observed (see Table 2).

Subsequently, after a number of fish-extract injections had been performed on the right arm, a corresponding reduction of local sensitivity occurred here also. After stopping the tests for 8 weeks the sensitivity had almost returned to its original level. It follows, therefore, that a kind of local immunization is possible; however, so far as our present experience goes, it is only transitory. We can not yet say whether it will be possible to immunize the whole body against the result of oral administration of the antigen. Experiments in this direction are contemplated.
CONCLUSIONS

1. According to our investigations on a highly fish-sensitive patient, fish supersensitivity exists both towards oral and intradermal administration of the antigen. The active principle is present in the muscle, but not in the serum and most of the organs of bony fishes, and only in small amounts in the muscles of cartilaginous fishes. It is not demonstrable in raw fish muscle but only develops on heating to the temperature of protein coagulation.

2. The active principle is insoluble in cold or warm alcohol and ether; it is non-dialysable, is speedily inactivated by acids, but not by alkalis, pepsin or trypsin.

3. The reaction is strictly specific. Precipitins, complement-binding and neutralizing antibodies were not demonstrable in the serum of the sensitive individual.

4. It was not possible to sensitize guinea-pigs passively with the patient's serum. But with his serum the specific transfer fish supersensitivity to normal, not fish-sensitive human beings was successfully accomplished. The technique consisted in the intradermal injection of the serum and re-injection of the same spot of skin, 24 hours later, with the antigen.

5. It was not possible with this technique to transfer sensitivity to pollen protein, horse serum or tuberculin. Perhaps we might have succeeded with the sera of persons more highly sensitive than those available to us.

6. After repeated performance of intradermal injections of fish extract there resulted a local reduction of sensitivity lasting for several weeks.