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STUDIES ON MOSQUITO BITES

By

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I. INTRODUCTION

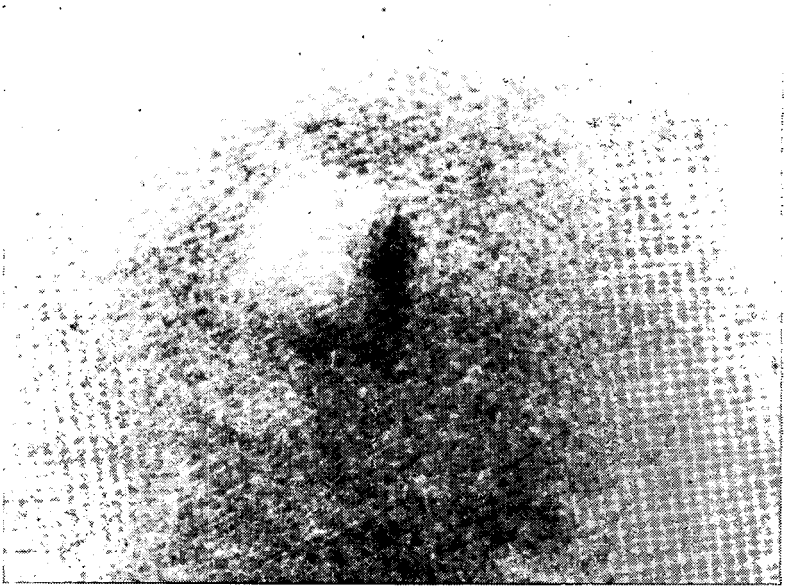
Because of individual qualitative as well as quantitative differences in the mode of reaction of man to mosquito bites, it has been generally assumed that mosquitoes have a special preference for certain individuals when they feed by sucking blood. *Gordon* (1922) and *Mellanby* (1946 b) have, however, shown by experiment that the mosquitoes attack all people indiscriminately if the experimental conditions are the same. The fact that some persons suffer particularly under the attacks of the mosquitoes must therefore be ascribed to peculiarities of the mode of reaction of these individuals.

The reactions of man to mosquito bites may manifest themselves in various manners. Either an immediate reaction may develop following the bite of the mosquito, consisting of a wheal and erythema, or the bite may be followed by a papular delayed reaction which will, as a rule, develop after 24 hours. The latter form of reaction may in exceptional cases assume the character of an Arthus phenomenon with necrosis (*Brown, Griffiths, Erwin & Dyrenforth*, 1938, *Culbertson*, 1941). Furthermore a peculiar flare-up of old sites following new bites of the same kind has been observed, the so-called "repetition reaction" (*Hase*, 1944). Finally, it should be mentioned that certain individuals do not react in any way after having been bitten by mosquitoes.

The immediate reaction is of the urticarial type, developing a few minutes after the bite, is transiently itching, reaching its maximum within 15 to 30 minutes, and subsiding within 30 to 60 minutes. The delayed reaction resembles a tuberculin reaction. It may persist for several days and is accom-

panied by a continual or intermittent itching. These reactions thus are analogous to the cutaneous reactions observed in allergic persons following intracutaneous injections of antigen to which these individuals have been sensitized; they have been characterized by *Sulzberger* (1940) as equivalent to these allergic reactions.

Biologists have for a long time attempted to explain the mechanism of the formation of a mosquito bite. *Cornwall & Patton* (1914-1915) thus mention



The figure shows the immediate reaction to *Aëdes aegypti* in a sensitized person (Exp. subj. No. 1) a few minutes after the bite (Magnif. $\times$ abt. 4).

that *Leeuwenhoek* in 1695 tried to explain the bite in a purely mechanical manner while *Reaumur* in 1738 is said to have presumed that the irritation in the skin is produced by the insect secreting a toxic fluid into it. *Schaudinn* (1904), on the basis of the finding of yeast fungi in the oesophageal diverticula of the mosquitoes, developed the theory that the skin reaction was produced by irritation by Carbonic acid formed by the enzymes of these micro-organisms, but *Cornwall & Patton* and *Roy* (1926-27) could not find yeasts in the digestive system of the mosquito. *Pawłowski* (1929) tries to show experimentally that the salivary secretion is the direct cause of the skin reaction, whereas *Roy* and *Manalang* (1931) find that substances with the same irritating effect are found not only in the salivary glands but also in other organs, even if in lower concentrations.

Hecht (1929 a & b, 1930, 1933) by experiment with *Anopheles maculipennis* shows that the immediate reaction must be of an allergic nature, since it is possible, by means of a modified Prausnitz-Küstner technique, to demonstrate circulating antibodies to the secretion excreted by the mosquito while sucking blood. The experiment, however, was only successful in two subjects. Attempts to demonstrate antibodies in individuals with delayed reactions failed. Even so *Hecht* presumed that the delayed reaction also depended on a sensitization, since an individual who has first given a negative reaction, later produces a delayed reaction.

Mellanby (1946 a) has provided further experimental support for the view that mosquito bites are allergic reactions. In experiments on *Aedes aegypti* and *Anopheles maculipennis* this observer finds that persons who have not been previously exposed to the bites of these mosquitoes, only exhibit delayed reactions; after having been bitten repeatedly for a month they present immediate as well as delayed reactions. After repeated bites the delayed reaction later becomes less pronounced, or it may disappear completely. Also the immediate reaction may subside in persons who have been exposed to the bites of *Aedes aegypti* over a long period of time. *Mellanby* suggests that in the mode of reaction of man to mosquitoes we are concerned, first with an increasing sensitization (no reaction $\rightarrow$ only delayed reaction $\rightarrow$ immediate reaction and delayed reaction), followed by a desensitization (immediate reaction and delayed reaction $\rightarrow$ only immediate reaction $\rightarrow$ no reaction).

These observations are supported by other experimental and practical experience. The literature contains several reports showing that after being exposed to a species of mosquitoes by which they have not been previously bitten, human beings at first react very strongly, later on in a lesser degree or not at all (*Dawson Williams* (1896-97), *Morse* (1896-97), *Nuttall & Shipley* (1901-03), *Hase* (1916), *Gordon* (1922) and *Pawlowsky* (1927)). Similar observations have been reported in the case of *Phlebotomus* by *Newstead* (1911-12), *Doerr & Russ* (1913) and *Boycott* (1928). The last-mentioned author finds immediate as well as delayed reactions in a person who has been previously exposed to bites by *Phlebotomus*, while 4 unexposed persons gave no reactions at first to the bites of this species of mosquito, but at the end of 12 days exhibit a typical response. Also after repeated bites of fleas or *Cimex lectularius* the capacity of reaction of man may be altered. *Boycott* (1912-13), seems to be the first to have observed this phenomenon by experiment with *Xenopsylla cheopis* and *Ceratophyllus fasciatus* (rat fleas). By experiment with *Spilopsyllus cuniculi* (rabbit flea) *Boycott* (1926) also found a sensitization (after 7 days) and for later bites the reactions developed after a latent period of 36 hours. *Kemper* (1929 and 1930) works with bed bugs. In an experiment on himself he allows about 30 bed bugs to suck blood from the same area of the skin (measuring about 4 cm. in diameter) every three days or so for about 11 months. At first the latent period for the appearance of a skin reaction, which is described as an "urtica", is 24 hours; at the end of 4

months, the reaction appears immediately, and at the end of 8 or 9 months, the subject gives no reaction at all. This condition is not, however, permanent, since reaction reappears after an interval of 4 weeks in the experiment, and despite repeated bites this reaction persisted for some time. *Kemper* (1929) in another subject found a latent period first of 7 days, but after repeated bites over a year the latent period was reduced to three hours. *Hecht* (1930) found circulating antibodies in a person who gave an intense immediate reaction after bites by bed bugs.

These observations and experiments—especially those described by *Hecht* and *Mellanby*—thus suggest that insect bites may be of allergic nature, i.e., the result of a sensitization, but it is considered necessary to study the course of the sensitization in greater detail. It is also an open question whether all reactions to mosquito bites are allergic. In my own work I have attempted to produce an altered mode of reaction (possibly a sensitization) by means of repeated bites over long periods of time, to demonstrate the presence of antibodies in sensitized individuals, and, finally, to evaluate the immediate and delayed reactions observed as an expression for an existing sensitization. Finally, systematic attempts at desensitization will be of theoretical as well as practical interest.

II. THE EXAMINATIONS HERE PRESENTED

The experiments have been carried out partly at the London School of Hygiene & Tropical Medicine (department of entomology), partly at the Royal Veterinary and Agricultural College in Copenhagen (zoological laboratory), and partly at the Children's Hospital, Martinsvej, in Copenhagen.¹

In the experiments *Aedes aegypti* (A.a.) have been used, which have been hatched and stored according to the directions given by *Christophers* (1947). During the part of the

¹ I am greatly indebted to Professor *P. A. Buxton*, Dr. *K. Mellanby*, Professor *Mathias Thomsen* and Dr. *A. Rothe-Meyer*, chief physician, for excellent working conditions and valuable suggestions and advice in the performance of the work.

work carried out in Copenhagen, the bubbling of air in the vessels with larvae and pupae has been omitted, and the food used was a special kind of fish food and Becoplex. At 28° C and 80 % relative humidity the first pupa was seen—as also found by *Christophers*—after 6 days, and the first adult mosquito hatched after 7 or 8 days. Like *Atkin & Bacot* and *Christophers* the author has made the observation that clean tapwater is unsuited for hatching the eggs, which were therefore placed in water from a pond in the garden of the Veterinary and Agricultural College. Even though mosquitoes which had formerly sucked blood from human beings or guinea-pigs, produced the same reaction at repeated bites, the insects used were practically all female mosquitoes which had never previously sucked blood.

When the mosquitoes were to be used in experiments, they were carefully caught in a small cylindrical glass (5 cm. long, 2 cm. in diameter), the open end being covered with wide-meshed gauze which was fastened to the sides of the glass by means of adhesive plaster or paper sealing strips. When thereafter the open end of the glass was held against the skin, the mosquito would pass its proboscis down through a gauze mesh (abt. 1 mm.²). The glass was pressed firmly against the skin (unless otherwise stated, on the volar aspect of the forearm), until the mosquito had again withdrawn the proboscis. The resultant reactions, were measured in mm., first in the longitudinal direction of the arm, and then in the transvers direction. The mosquitoes generally fed for 2-5 minutes, only in exceptional cases from 5 to 10 minutes. The reactions were read immediately after removal of the glass, and further after 15 and 30 minutes. Unless expressly stated, "immediate reaction" in the following means the size of the skin reaction 30 minutes after the bite of the mosquito. In practically all the cases the reactions have been inspected at the end of 24 hours.

41 persons were used as experimental subjects (in the following called E.S.), one of these persons both in Copenhagen and in London (experiments carried out on the writer), 15

persons in London only and 25 persons in Copenhagen only. Of the latter, 10 were children, aged 6 months to 3 years, average 20 months. Of the adult subjects, 7 were women and 24 men, aged 18 to 55 years, the average age being about 30 years. A complete list of the experimental subjects is given in Table 1.

TABLE I
List of experimental subjects
(the number indicates age in years).

1. H. ♂ 34	11. A. ♂ 32	21. J. ♂ 20	31. W. ♂ 20
2. M. ♂ 39	12. J. ♂ 31	22. T. ♀ 41	32. W. ♂ 6 months
3. H. ♀ 32	13. W. ♂ 34	23. A. ♀ 19	33. C. ♀ 16 months
4. S. ♂ 47	14. L. ♀ 30	24. H. ♂ 18	34. N. ♂ 14 months
5. L. ♂ 35	15. H. ♂ 37	25. A. ♂ 19	35. P. ♀ 13 months
6. C. ♂ 30	16. M. ♀ 20	26. M. ♀ 20	36. W. ♂ 2
7. L. ♂ 55	17. J. ♂ 45	27. J. ♂ 20	37. T. ♂ 1
8. B. ♂ 32	18. T. ♂ 51	28. C. ♂ 19	38. J. ♂ 18 months
9. W. ♂ 27	19. J. ♂ 19	29. H. ♂ 18	39. J. ♀ 2
10. M. ♀ 25	20. L. ♂ 40	30. F. ♂ 19	40. P. ♂ 3
			41. M. ♂ 15 months

Extracts were made either of mosquitoes which after having been killed with chloroform vapour had their wings and legs cut off, or of salivary glands which had been dissected out. The material was first placed in an exsiccator for 24 hours at 35° C., weighed and ground with powdered glass dust. After extraction with ether the non-lipoid remainder was suspended in physiological salt solution with 0.5 per cent. phenol, shaken for one hour and placed in an incubator for 48 hours. Next the supernatant fluid was pipetted off, filtered through a Seitz filter with a water jet pump and subjected to a sterility test. In extracts of whole mosquitoes the concentration was about 1/50. An amount of 0.1 cc. of the extract contains material corresponding to one mosquito. For the salivary extract, glands from 33 adult female mosquitoes were used (amount of extract 3.3 cc., i.e., 0.1 cc. of extract corresponds to the salivary glands of one mosquito).

B. *Experimental Results.*

1. *Experiments carried out on the Writer.*

The experiment started on May 13th, 1947. During the first 16 days the experimental subject (E.S. No. 1) was ex-

posed to 5 bites of A.a. daily. The subject had not been previously exposed to the bites of this species of mosquito which does not occur in northern Europe. During the first few days only small non-itching papules were seen 48 hours after the bite (abt. 3 mm.). On the 9th experimental day larger reactions appeared at the sites of two bites which were 48 hours old (measuring 8×8 and 13×13 in diameter), and on the 10th day reactions manifested themselves at 4 of the five sites of punctures which were 48 hours old (average size 13×12 mm.) and on the 11th day all five spots bitten reacted after 48 hours (12×12 mm.). Simultaneously with the development of these delayed reactions the subject felt an intense itching, which had not been the case after the bites during the first 8 days. After the 12th day the reactions declined quickly at the spots on which bites had been received 48 hours previously, and after the 17th day only small, not-itching papules were seen 48 hours after the bites.

On the 9th day, however, delayed reactions were also observed at the 24-hour sites of puncture where the reactions had formerly been negative. The delayed reaction after 24 hours on an average measured 7×8 mm. and were itching. On the 10th day the average measurement was 8×8 , on the 11th day 9×9 , and on the 12th day 12×13 mm. From the 12th to the 16th day the average of the daily 5 bites after 24 hours was 14×14 mm.

On the 15th day immediate reactions suddenly arose (wheal 7×6 mm., erythema 35×33 mm.) at the spots bitten: immediately after the mosquitoes had finished feeding these became the seat of intense itching.

From the 17th to the 58th day of the experiment the subject only had one bite daily (or, in some few cases, every two days). The delayed reaction after 24 hours from the 17th to the 40th day measured 10-20 mm., with an average of 14-15 mm. On the 42nd day the delayed reaction suddenly appeared after a latent period of only about 12 hours, and during the remaining part of the experimental period the latent period was gradually reduced to 8 or 9 hours, and

the reaction increased to 19-45 mm., the average size being 26×26 mm.

After an interruption of two months the experiment was resumed, one bite being administered every two days in the period September to November 1947 (105 days). During this period the immediate reaction consisted of a wheal measuring on an average 8×6 mm., and an erythema of 42×44 mm.

During this second experimental period the delayed reactions at first appeared after a latent period of 8 hours, but eventually at the end of only 2 or 3 hours. The average size of the delayed reaction was 15×15 mm. (8-25 mm.). Generally a small vesicle was observed at the site of puncture 24-48 hours after the bite.

Early as well as late reactions were accompanied by itching: the early reactions were of rather short duration, while the delayed reactions might itch rather intensely for a couple of days. Occasionally old bites would flare up when a new reaction to a bite developed in their vicinity or when the region was exposed to the influence of heat or friction. In experiments with bites on the thigh and abdomen the reactions were of approximately the same size as those observed on the forearms, except that the erythema was slightly larger.

The rather sudden reduction of the latent period on the 42nd day of the first experimental period occurred 8 days after injection with A.a. extract had been started; it will be dealt with in some detail later in this paper.

2. *Experimental Subjects who have been previously exposed.*

The exposure to bites by A.a. is designated as *intense* when the subject has been bitten many times over a long period of time, as of *medium* strength when the subject has only been exposed to bites for a fairly short time, and as *weak* if the subject has previously only been bitten a few times by A.a.

This group comprises 14 persons, 4 of whom have been intensely exposed (I), 5 submitted to exposure of medium

strength (II), while 5 persons have been only weakly exposed to bites by A.a. (III).

Of the 4 persons in group I, two presented no reaction (E.S. Nos. 4 and 15), while 2 displayed immediate reactions only (E.S. No. 5, wheal 5×5 , erythema 40×50 —E.S. No. 11, wheal 8×9 , erythema 40×50). Of the 5 persons in group II 3 exhibited immediate as well as delayed reactions (E.S. No. 2, wheal 6×5 , erythema 35×35 , delayed reaction (read at the end of 20 hours) 20×20 —E.S. No. 7, wheal 7×7 , erythema 40×50 , delayed reaction (read at the end of 24 hours) 20×20 —E.S. No. 16, wheal 10×10 , erythema 55×62 , delayed reaction (read at the end of 24 hours) 22×27 mm.) and 2 subjects only immediate reactions (E.S. No. 8, wheal 7×6 , erythema 50×70 —E.S. No. 12, wheal 12×6 , erythema 40×50 mm.).

Of the 5 persons who were weakly exposed (group III), 3 responded with immediate as well as delayed reactions (E.S. No. 6, wheal 8×5 , erythema 40×40 , delayed reaction (read at the end of 24 hours) 50×45 —E.S. No. 9, wheal 12×9 , erythema 35×40 , delayed reaction (at the end of 24 hours) 23×43 mm.—E.S. No. 14, wheal 18×7 , erythema 35×75 , delayed reaction (read at the end of 24 hours) 50×60 mm.; one responded with an immediate reaction alone (E.S. No. 10, wheal 4×5 , erythema 30×50 , and one with only a delayed reaction (E.S. No. 13, 9×7 mm., with a central vesicle).

The two subjects who had been intensely exposed and who gave no reaction (Nos. 4 and 15) were exposed to several experiments with bites. E.S. No. 4, who was a native of India and throughout his life had been exposed to "thousands of bites" received altogether 9 bites within 11 days, but it was not possible to alter his reaction. An experiment carried out 3 weeks later also gave a negative result. On the other hand, E.S. No. 15, who did not react to the first bite, developed weak, itching reactions to 2 bites on the 2nd experimental day (wheal 8×8 , erythema about 22×32 mm.). We are hardly concerned with any sensitization in this case, and this subject

should undoubtedly be included in the group of primarily occurring immediate reactions.

Attempts to procure immediate reactions in E.S. No. 13 by repeated bites over a period of 15 days failed. An urticarial wheal measuring 7×7 mm. was indeed observed on the 6th day, but there was no erythema and neither before, nor later did this subject exhibit the slightest indication of an immediate reaction.

A survey of these experiments is given in Table II.

TABLE II
14 adult experimental subjects previously exposed.

Reaction before experiment	Number	Exposed to repeated bites	Reaction altered By experiment
No reaction (absolute anergy)	1	1	0
Immediate reaction only	6		
Immediate & delayed reaction	6		
Delayed reaction only	1	1	0
No reaction (relative anergy)	0		

3. *Experimental Subjects who have not been previously exposed to A.a.*

a. *Adults.*

The experiments comprise 16 persons. 4 subjects (I) gave no reaction to the first bite, while a delayed reaction alone appeared in five persons (II), and 4 persons (III) only presented immediate reactions and three persons (IV) immediate as well as delayed reactions.

I. E.S. Nos. 3, 17, 20 and 24 gave negative reactions to the first bites. E.S. No. 3 was bitten once daily by one mosquito. The first two days only small palpules measuring a couple of mm. were in evidence, but on the third day a delayed reaction measuring 12×12 mm. appeared at the 48-hour site of puncture, and at the 24-hour site a reaction of 8×8 mm. After the experiment had been going on for 10 days the delayed reaction measured 15×17 at the end of 24 hours, and after 48 hours 10×10 mm., but there was no immediate reaction.

E.S. No. 17 presented on the 4th day (at the 2nd bite) an immediate reaction which it was possible to reproduce in the following four experiments, but a delayed reaction never appeared. The possibility cannot be ruled out that this subject had been exposed to bites of mosquitoes previously to the experiment, as he assisted the writer in hatching the mosquitoes. Therefore he should be left out of the material.

E.S. No. 20 was bitten every second day, and on the 17th day a delayed reaction developed at the end of 24 hours (8×10 mm.). The delayed reaction continued to be of the same size during the following two weeks, and there was no immediate reaction.

E.S. No. 24 was bitten every three days for 18 days and remained without reaction.

II. E.S. Nos. 21, 22, 26, 29, and 31 developed delayed reactions at the first bite. No more experiments were conducted on E.S. Nos. 22 and 26. In E.S. Nos. 21 and 31 continued bites produced an immediate reaction. The delayed reaction in E.S. No. 21 measured 10×7 , increasing till it measured 33×29 on the 12th day (with a vesicle 3 mm. in diameter centrally). The immediate reaction in this subject appeared after 4 exposures and on the 12th day. The wheal measured 8×8 , the erythema 30×30 mm., 13 days later a similar reaction was observed (wheal 5×6 , erythema 28×28). In E.S. No. 31 the delayed reaction at first was 10×10 and attained approximately this size during the remaining part of the experiment. The immediate reaction appeared on the 18th day and at the 7th exposure. The wheal measured 6×5 , and the erythema 25×20 . An experiment on the following day gave approximately the same reaction.

E.S. No. 29 was bitten 8 times within 24 days, in the course of which time the delayed reaction increased from 10×10 to 25×15 mm., but no immediate reaction appeared.

III. E.S. Nos. 18, 19, 27, and 30 at once exhibited immediate reactions (average size of wheal 8×7 , of erythema 34×43).

IV. E.S. Nos. 23, 25, and 28 at once presented immediate reactions as well as delayed reactions. In E.S. Nos. 23 the wheal (after 15 minutes) measured 18×14 mm., the erythema 40×50 mm., the delayed reaction (after 24 hours) 60×40 mm.; in E.S. No. 25 the wheal was 8×7 mm., the erythema 50×50 , the delayed reaction (after 24 hours) 30×30 mm., and in E.S. No. 28 the wheal (after 15 minutes) was 5×6 and the erythema 35×40 while the delayed reaction was somewhat varying at four exposures (in two experiments from 2 to 4 mm., in the other two experiments 8×11 and 12×12 mm. respectively).

E.S. Nos. 23 and 28 were allergic persons (previous urticaria, bronchial asthma and allergic eczema). The other subjects had not previously suffered from allergic conditions and could give no information about any predisposition. All reactions and results of experiments in this group are given in table III.

TABLE III
15 adult experimental subjects not previously exposed.

Reaction before experiment	Number	Exposed to repeated bites	Reaction altered by experiment	Reaction after experiment
No reaction (absolute anergy)	0			0
Immediate reaction only	4			4
Immediate & delayed reaction	3			5
Delayed reaction only	5	3	2	5
No reaction (relative anergy)	3	3	2	1

b. Children.

None of the ten Danish children aged 6 months to 3 years (average age 18 months) presented any reaction to the first bite, 4 of them were exposed to repeated bites, and all four gradually developed delayed reactions (cf. Nos. 32, 34, 35, and 36).

In E.S. No. 32 the reaction was observed for the first time on the 23rd day at a spot which had been bitten 24 hours previously (15×10 mm.). After 48 hours the reaction was 15×15 mm. The alteration in the skin's capacity to react appeared after 8 bites. In E.S. No. 34 a reaction measuring 7×8 mm. was seen on the 9th day at the 48-hour site of puncture, and a papule, 3×4 mm., with a central vesicle at the 24-hour site. During the following days delayed reactions kept appearing after 24 hours, but nothing could be seen at the site of puncture immediately after the bite. The delayed reaction at first increased in size 24 to 48 hours following the bite, but from the 14th day it attained its maximum size within 16 to 24 hours following the bite. The size on the 14th day was 13×15 , on the 16th day 16×17 and on the 23rd to the 24th day 22×15 mm. The positive reaction appeared after 6 exposures. In E.S. No. 35 reaction measuring 5×5 mm. appeared on the 6th day at the 48-hour site of puncture, and on the 7th day a reaction (25×25 mm.) at the 24-hour site. The positive reaction appeared after 4 exposures.

E.S. No. 36 suddenly exhibited a delayed reaction (8×8 mm.) at a 24-hour site on the 14th day of the experiment. The reaction could be reproduced in an experiment 2 days later (6 mm.).

4. *Experiments with Extracts of A.a.*

8 persons who had formerly been exposed to bites of *A.a.* were given intracutaneous injections of 0.1 cc of whole-mosquito extract made along the lines previously mentioned in this paper. The experiment includes E.S. Nos. 2, 4, 6, 10, 13, 14, 15, and 16. Only in one person, viz. E.S. No. 15, a positive reaction was observed, the wheal increasing from 6×5 to 11×10 mm., the erythema measuring 20 mm.; the reaction itched the first 10 minutes, and there were distinct pseudopods. In all the other persons the intracutaneous test had a negative result. Attempts to demonstrate circulating antibodies in E.S. No. 15 by means of Prausnitz-Küstner's technique failed.

Also in E.S. No. 1 a definitely positive, but weak intracutaneous reaction was demonstrated. The initial wheal was 7×6 mm. and without erythema. Within 20 minutes a typical immediate reaction with pseudopods (wheal 12×10 , erythema 30×25) developed, accompanied by a good deal of itching; after 24 hours a red infiltration (7×5 mm.) was in evidence. This result of the intracutaneous test could be reproduced. The initial wheal increased from 6×5 to 13×9 mm. within 30 minutes; the erythema was 25×35 mm., the infiltrate after 24 hours 15×15 , red and itching. Also with serum from this subject the Prausnitz-Küstner experiment gave a negative result.

Both of the two subjects who gave positive reactions must be considered to have been intensely exposed to *A.a.*, E.S. No. 15 having stayed for many years in tropical countries where the *A.a.* is found, while E.S. No. 1 had the intracutaneous tests done on the 30th and 50th days of the experiment.

Like the other subjects, E.S. Nos. 1 and 15 gave negative reactions to whole-mosquito extract from which the fat had not been removed with ether, and to salivary gland extract produced as described above.

5. *Experiments with Inoculation of Fresh Organs.*

Experiments with intracutaneous inoculation of crushed, fresh salivary glands or intestine gave varying results. Fresh salivary glands from one mosquito, crushed and suspended in physiological salt water with $\frac{1}{2}$ % phenol produced in E.S. No. 2 considerable swelling after about 24 hours. An area measuring 50×50 mm. surrounding the site of inoculation was red and itching. A central infiltration measuring 9×12

mm. was felt. Nothing was visible immediately following the inoculation. In contrast to this, salivary glands which had first been dried and thereafter suspended in physiological salt water with phenol, produced an "immediate reaction" which, 30 minutes after the inoculation consisted of a central area of the same size as the initial wheal (8×9 mm.) and an erythema of 30×50 mm. 24 hours later a central infiltration measuring 9×10 mm. was seen surrounded by an erythema of 40×60 mm. The reaction subsided within the next 24 hours. The same subject presented after inoculation of intestines, etc., an "immediate reaction" which after 30 minutes measured 20×20 mm., the central infiltration being of the same size as the initial wheal. 24 hours later it was 10×20 mm., and the erythema 40×40 . Control injections with physiological salt solution with $\frac{1}{2}$ % phenol alone gave negative results. The above mentioned "immediate reactions" had an urticarial appearance, while at the end of 24 hours a swelling was in evidence which was of an inflammatory type, red, itchnig intensely, often painful. These skin reactions bore no resemblance to the reactions following bites or the positive intracutaneous reactions obtained with extract of A.a.

III. DISCUSSION

A remarkable result of the studies here presented is the demonstration of an initial period without reaction (in the following called relative anergy period) followed by the appearance of a papular delayed reaction at continued exposure to the bites of the mosquito. This relative anergy period has formerly been observed by *Hecht* in an isolated case. *Mellanby's* subjects, on the other hand, all of them at once responded with a delayed reaction, and *Tezner* (1934) claims that practically all persons reacted to mosquito bites with swelling and erythema. In the experiments here recorded $\frac{4}{5}$ of the adult Danish persons who have never previously been bitten by A.a. at once exhibited delayed and/or immediate reaction (see Table III), whereas all ten children, aged

from abt. 6 months to 3 years, gave negative reactions, and in the four cases, in which the children were exposed to bites for some weeks, delayed reactions were produced after a period of from 6 to 23 days. *Tezner* (1934) stresses that *Hecht's* and *Kemper's* observations (see the introduction) are in favour of a view according to which the reactions of man to insect bites are comparable to the results of sensitization of human beings and other mammals by intracutaneous injections of foreign serum and ovalbumin. *Tezner* by experiments with ovalbumin (1934 a and b, 1935), found that after a relative anergy period of one week a delayed reaction, and one week later, also an immediate reaction set in. The former might disappear within 2 or 3 weeks, but in several cases it persisted unchanged. In some of the cases, but not in all, the immediate reactions also disappeared after continued intracutaneous injections of ovalbumin. By means of the Prausnitz-Küstner technique it was possible to demonstrate the presence of circulating antibodies.

If *Tezner's* observations are correlated with those of *Hecht*, *Mellanby* and my own, reported here, the resemblance of the form of reaction as to type and sequence is so striking that it would seem safe to presume that we are concerned with analogous processes. It is true that I have not been able to demonstrate the presence of circulating antibodies, as *Hecht* has done, but it is a well-known fact that the failure to demonstrate antibodies does not rule out the possibility of a sensitization: it is possible that the antibodies against the antigens in the salivary secretions of the mosquito may be present only in the skin.

The certainly weak, but definitely specific positive intracutaneous reactions which I observed in 2 subjects following injection of extract of A.a., also support the view that these individuals have become sensitized to A.a.

The fact that abt. 80 per cent. of the adult individuals who have never previously been exposed to the bites of A.a. respond with a delayed and/or immediate reaction, however, calls for a more detailed discussion of the question of the

allergic nature of the mosquito bites. This observation seems to support the presumption that the reaction of the human being to the bites of mosquitoes involves a primary toxic (possibly urticariogenic) influence. It is a well-known fact that intracutaneous injection of any fluid may elicit an immediate as well as a delayed reaction in the skin by a physical or chemical irritation (*Tezner*, 1935). Taking into account the infinitely small amount of fluid which the mosquito secretes when biting, a physical influence may presumably be left out of account. It is not possible, however, a priori to rule out the possibility of a primary toxic irritation by the saliva of *A.a.*, but it is rather unlikely that such a toxic irritant really does exist.

First of all, the possibility can hardly be excluded that the above mentioned Danish subjects who reacted to the first bite, may have been previously sensitized, e.g., by having been bitten by Danish mosquitoes. All Danish Culicidae are bloodsucking (*Wesenberg-Lund*, 1920-21). Species of *Aedes* occur among them and they frequently bite man. If, therefore, a person has not been out of Denmark or England he will not have been bitten by *A.a.*, but he will probably have been bitten many times by very closely related species. Antigens partaking of the character of haptens may possibly be of significance in this connection.

The question of the specificity of the sensitization produced by the mosquitoes has yet to be determined. Subject No. 2 reacted to bites of *A.a.* with an immediate as well as a delayed reaction, but gave a negative reaction to bites of *A. albopictus*, E.S. No. 10 responded with immediate reaction to *A.a.* but was without reaction to *albopictus*, and E.S. No. 16 reacted to both species of *Aedes* with immediate as well as a delayed reactions. Also in E.S. No. 1 an immediate as well as a delayed reaction of identical intensity to these two species of mosquitoes was found on the 22nd day of experiment, but the subject had never previously been bitten by *Aedes albopictus*. At the same time this subject was without reaction to *Anopheles maculipennis*, *Culex molestus*

and Cimex. As will be seen, nothing definite as to the specificity of the sensitization can be concluded from the examinations here presented.

Secondly, if the view is insisted that the secretion of the mosquito has a primary toxic effect, it will indeed be difficult to explain that a number of the subjects give no reaction whatsoever to the first bite, but begin to react after a certain latent period. The experiments on children suggest strongly a sensitization. In this connection, it is of interest that *Sulzberger* (1940) should stress that "many extracts considered as primary irritants solely because of the high incidence of reactions produced in adults, when applied in identical concentrations and manner elicit no whealing whatsoever in normal infants". Applied to the observations here presented this view involves that the primary reactions in a number of the adult subjects need not offer any obstacle for a presumption taking the mosquito bites to be of allergic nature, since the children have given negative reactions, but in several cases by repeated exposures have become enabled to react in the same manner as adult persons. The primary reactions in adult individuals thus only become an expression for the degree of sensitization of these persons.

The experiments on sensitization which have been carried out by Hecht, Mellanby and in the studies here presented, will thus have to be taken as very essential arguments in favour of the view that the mode of reaction of man to mosquito bites depends on the degree of sensitization of the individual. In this connection it may be indicated that a comparison of Table II and III shows that the subjects in Table III are definitely less sensitized than the subjects in Table II, but it is seen that by experiments they have been brought to a higher degree of sensitization.

Respecting the type of and relation between the immediate and delayed reactions observed, it should be stated that the results of the examinations on these points are in keeping with the experiences from other allergic reactions. The delayed reaction is changed by repeated biting, while the immediate

reaction is difficult to influence. *Mellanby* has shown that the delayed reaction may disappear completely, and in the present examinations (E.S. No. 1) its latent period was shortened significantly. These observations are also in harmony with *Kemper's* experiments, about which *Tezner* (1934 a) said that one cannot help thinking of the alteration of an allergic delayed reaction into an immediate reaction, in the same manner as it has been observed in his own sensitization experiments with ovalbumin. Such a change-over from a delayed to an immediate reaction has also been described by *Marcussen* (1948) as far as the reaction to trichophytin is concerned. *Mellanby's* examination as well as the experiments here presented seem to suggest that the reactions following the bites of insects take a similar course.

While *Mellanby* observed that individuals by repeated exposure to mosquito bites may become entirely negative (absolute anergy), *Benson* (1936) by desensitization of patients to mosquito bites finds that delayed reactions may be caused to disappear while the immediate reaction persists. The latter finding is in keeping with the experiences from other forms of treatment with desensitization, but the possibility cannot be ruled out that there may be a difference between treatment with extracts and the effects of numerous, frequently repeated bites over long periods of time. As mentioned in the introduction, practical experience supports the view that man may become entirely immune to mosquito bites. Such an absolute anergy is also in evidence in E.S. No. 4, who is a native of India and has lived his whole life in places where he has been frequently exposed to bites of *A.a.*

The conclusions appears to be that, at least so far as the delayed reactions to mosquito bites are concerned, a specific desensitizing treatment should greatly reduce the inconvenience caused to the individual.

After this discussion of the theoretical aspects, some few observations may be set down.

The primary anergic period has been demonstrated in 7 cases (in E.S. Nos. 1, 3, 20, 32, 34, 35, and 36). Regard-

ing E.S. Nos. 1, 3, and 34 it has been stated previously that a simultaneous reaction is observed at the 48-hour and 24-hour sites of punctures on the day when the change sets in. In all 3 subjects the former reactions are the largest. The same findings are observed on the following days; my conclusion is that the latent period is 24 hours but that the reaction increases in size in the period 24 to 48 hours following the bite. In the experiments described, after a few days the reaction reached its maximum size about 24 hours after the bite.

The length of the primary anergic period is on an average 11-12 days. The period of time required to establish sensitization to the salivary secretion of the mosquitoes is thus of practically the same duration as in the case of sensitization to other antigens.

Immediate reaction was produced experimentally in E.S. Nos. 1, 21, and 31, the two latter of whom reacted primarily by a delayed reaction, while E.S. No. 1 had a relative anergy period of 8 to 9 days. The immediate reaction made its appearance 15, 12, and 18 days respectively (average 15 days) after the beginning of the experiments. It is thus possible that the sensitization period before the appearance of the immediate reaction is independent of the primary mode of reaction of the individual, but of course nothing definite can be concluded on the basis of such a small number of persons.

Attempts to produce immediate reactions failed in two cases (E.S. Nos. 13 and 29, who were bitten for 15 and 24 days respectively), while attempts to produce a delayed reaction in a primarily anergic subject only failed in one case (E.S. No. 24).

In the discussion of the latent period of the delayed reaction in E.S. No. 1, stress is put on the sudden reduction of this period observed about 8 days after the institution of a desensitizing treatment with A.a.-extract. Following injection of increasing doses of this extract administered superficially subcutaneously for three weeks, the latent period is only about 8 hours, and 2 months later the latent period is found to be of the same length. At the end of the 2nd series of experiments

the latent period is only 2-3 hours. While the delayed reaction increased very gradually in size during the first half of the experiment, it declined in the second half of the experimental period, but did not go below the level it had at the beginning of the first series. Thus the attempt to bring about a complete desensitization of E.S. No. 1 with regard to the delayed reaction did not succeed, but, as already mentioned, the experiment calls to mind *Kemper's* investigations and presumably illustrates a transition from the stage in which there was an immediate as well as a delayed reaction to the occurrence of only an immediate reaction following the bite.

Tezner (1935) found that allergic persons (and persons with an allergic predisposition) in sensitization experiments react with a primary immediate reaction, but that it is possible to elicit a delayed reaction in these persons later on. It is striking in this connection that of the three Danish persons who presented immediate as well as delayed reactions the two had a history of previous manifest allergy. The other subjects could state nothing about allergy.

Comparative studies on the effect of bites of A.a. and of Danish mosquitoes have not been carried out, and would probably be of very little value because of the lacking specificity previously mentioned. By questioning the Danish adult experimental subjects about the inconvenience caused to them by Danish mosquitoes it would seem that a parallelism exists between the degree of the capacity to react to A.a. and to Danish mosquitoes. 9 subjects with a primary anergic period or primary delayed reactions to A.a. have noticed slight or no reactions following bites of Danish mosquitoes, while 2 of 4 persons with primary immediate reactions complained of fairly considerable inconvenience from the latter. Finally, all three persons who responded to A.a. with immediate as well as delayed reactions stated that they were greatly inconvenienced by the bites of Danish mosquitoes.

It is of considerable interest, theoretical as well as practical, that the two positive cutaneous reactions were obtained by means of an ether-treated extract, while an extract of the

same concentration, but without ether-extraction of the fats always gave a negative reaction. After evaporation of the ether extract a thick fatty layer was in evidence. This result confirms the experience that the presence of large amounts of lipoids will inhibit the effect of the antigens present in the extract.

The experiments on inoculations of organs are difficult to evaluate, the sterility is hardly complete, the reactions ill-defined, of an inflammatory character. These reactions are hardly comparable to the very different, well-defined and constant skin reactions following bites or injection of extract.

Both *Sulzberger* (1940) and *Urbach* (1944) mention the bullous reaction which certain individuals, especially children, exhibit following bites of insects. In the present experiments vesicular reactions (formations of a vesicle centrally on the papular delayed reaction) were observed in 5 subjects (Nos. 1, 21, 34, and 35, who had all of them been sensitized experimentally, and in No. 7 who had been intensely exposed to the bites of A.a.). A comparison of the reactions produced in E.S. No. 16, partly by A.a. and partly by *Aedes albopictus*, showed a severe bullous reaction (bulla 5 mm., infiltration 20 mm.) to the latter mosquito, whereas the delayed reaction following bites of A.a. was exclusively papular (22×27 mm.). The immediate reaction to *A. albopictus* was weaker than to A.a. (wheal 6×6 and 10×10 respectively, erythema 25×25 and 62×55 mm. respectively). E.S. No. 16 was examined by means of extracts of the two species of mosquitoes but reacted negatively to both.

As regards the so-called "flare-up" reaction which was mentioned in the introduction, it should be noted that it was apparently observed in E.S. No. 1 but that the phenomenon was produced not only by the development of new bites but also by rubbing or the influence of heat on the skin in the vicinity of old bites. The "flare-up" phenomenon, therefore, can hardly, as far as mosquito bites are concerned, be adduced as an argument for the presence of a local specific sensitization of the sites of puncture.

CONCLUSION AND SUMMARY

Mosquito bites produce immediate and delayed reactions equivalent to those elicited by allergic intracutaneous tests. *Hecht's* and *Mellanby's* experimental studies support the view that the reactions of man to mosquito bites are due to a sensitization.

In the present experiments *Aedes aegypti* have been used. Experiments were made on the writer and 40 other persons, including 10 children. Repeated exposures and intracutaneous tests with extracts of mosquitoes have afforded further support for the theory of an allergic nature of the mosquito bites. The experiments also suggest the value of desensitizing therapy but only as far as the delayed reaction is concerned.

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