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INFLUENCE OF REPEATED AND LOW DOSES OF PERTUSSIS VACCINE ON HISTAMINE SHOCK

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The mouse and rat are animals which show a considerably high resistance to histamine. To produce histamine shock in these animals, considerably high doses of histamine are required. The high resistance of these animals can be reduced, however, by a variety of means. Thus, it was shown that the resistance of these animals is reduced by adrenalectomy (*Halpern & Wood*, 1950), implantation of sarcoma 180 (*Parfentjev*, 1955), advanced age (*Parfentjev*, 1955b), administration of pertussis vaccine (*Parfentjev & Goodline*, 1948), infection by *B. pertussis* (*Pittman*, 1951). To reduce the resistance to histamine by means of the pertussis vaccine, relative high doses of this vaccine are used (*Kind*, 1958). Yet, in most cases these high doses have a toxic effect on the organism. It is presumed therefore that routine pertussis vaccination is not able to reduce the resistance to histamine in children since the doses used for the vaccination of children are much lower than those which produce sensitization to histamine in mice and rats. On the other hand, it was proved recently by *Sanyal* (1961) that children examined after pertussis infection or pertussis vaccination are more susceptible to histamine than healthy, unvaccinated children.

Since it had been found that the low doses of pertussis vaccine do not produce sensitization to histamine in mice and rats, studies were undertaken to test the response of these animals to the repeated administration of pertussis vaccine. It is known,

indeed, that the repeated pertussis vaccination does not produce potentiation of anaphylactic shock in the mouse (*Kopytovskaya*, 1958, *Chedid & Boyer*, 1960), but this was reported only in cases where the first injection induced an increased formation of pertussis antibodies. Similarly, the passive administration of an antiserum which contains antibodies against some *B. pertussis* antigens (not agglutinins) inhibits the development of sensitization after pertussis vaccine administration (*Maitland et al.*, 1955).

The aim of the present study was to determine the effect of low and repeated doses of pertussis vaccine on the course of sensitization produced by this vaccine.

METHODS

The vaccines were prepared from fresh strains controlled as to the properties of the I. phase. The strains were multiplied on fluid media either by the shaking or the submerged culture method. After attaining a density of about 80×10^9 microbes per 1 ml. the 16–20-hour cultures were killed by adding 0.2 % of formol to them. In experiments reported in the present study the vaccines 490/S₁, 595/S₂ and 1945/57 were utilized.

The density of vaccines was determined on a Pulfrich nephelometer by comparing with the international turbidity standard (International Biological Standard held by Statens Serum Institute, Copenhagen Denmark).

Histamine shock was produced by intraperitoneal injection of histamine (Histaminum dihydrochloricum Spofa). In mice the shock was evaluated by the number of animals dying within 4 hours following the administration of histamine. In rats the course of shock was tested by measuring rectal temperature with a registration thermistor thermometer or, in some cases, with a quick-operating mercury thermometer. The animals were fixed in special boxes at a room temperature of 20° C.

Desensitization with histamine was carried out by repeated intraperitoneal injection of 5 mg histamine to rats. Each animal received the total of 5 injections given every day.

Desensitization with endotoxin (prepared from *S. paratyphi* B) was carried out by 5 intraperitoneal injections given every day.

RESULTS

The study was primarily concerned with the determination of the time necessary for the development of sensitization to histamine after various doses of pertussis vaccine. The dura-

tion of sensitization after the administration of various doses was determined concurrently. The results of these experiments are shown in Table I. From this table it follows that sensitization does not develop—even after high doses of pertussis vaccine—in less than 48 hours. The peak of sensitivity is found to occur after 5 to 14 days and depends on the height of the sensitizing dose employed. No signs of a lowered resistance to histamine were found after the administration of low doses.

In the subsequent studies our interest was concentrated on the effect of the repeated doses of pertussis vaccine on sensitization. For this purpose the mice were immunized with the vaccine 490/S₁, in the dose of 5×10^9 , given intraperitoneally in 0.5 ml. At definite time intervals the animals were then given the second dose, 0.5×10^9 and 5×10^9 respectively. After, respectively, 24 hours and 5 days following the second dose the mice were injected with histamine. For purposes of antibody titer determinations a group of mice were sacrificed by exsanguination immediately before the administration of the second vaccine dose to the remaining mice. This experiment was difficult owing to the fact that the administration of the second dose (5×10^9) produces in mice a shock which is lethal if the second dose is administered within less than 28 days. Due to this known fact it proved necessary to utilize in this experiment a major number of animals. For this reason the vaccine was also administered in the dose of 0.5×10^9 which did not produce lethal shock. The results of these experiments are shown in Table II. From this table follows that the second pertussis vaccine dose produces an apparent aggravation of the histamine shock if histamine is administered after 24 hours following this second dose. The effect of repeated administration was apparent for both doses, 5×10^9 and 0.5×10^9 respectively. The administration of histamine on the 5. day following this second dose failed in most cases to demonstrate the already enhanced effect of this second sensitizing dose, not even at a time by which the effect of the first dose had subsided, i.e. after 20 days. On the contrary, it seems

TABLE I

Decrease in resistance to histamine after various doses of pertussis vaccine 595/S₁.

Dose of vaccine	Number of animals succumbed after the administration of histamine at various intervals from the sensitization								
	24 hours		48 hours		5 days		14 days	21 days	28 days
	1 mg	5 mg	1 mg	1 mg	1 mg	5 mg	1 mg	1 mg	1 mg
100 × 10 ⁶	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
500 × 10 ⁶	0/10	0/10	0/10	0/10	1/10	1/10	2/10	1/10	0/10
1,000 × 10 ⁶	0/10	0/10	0/10	0/10	2/10	4/10	2/10	0/10	0/10
2,000 × 10 ⁶	0/10	0/10	4/20	0/18	9/26	5/18	5/10	1/10	0/10
4,000 × 10 ⁶	0/10	0/10	7/19	9/18	7/11	8/10	7/10	4/24	1/10
8,000 × 10 ⁶	0/10	0/10	—	—	9/10	8/10	8/11	—	—
10,000 × 10 ⁶	0/10	0/10	5/10	8/19	10/10	10/10	10/10	—	—
20,000 × 10 ⁶	0/10	0/10	4/10	8/10	10/10	10/10	—	—	—

TABLE II

Susceptibility to histamine after the second dose of pertussis vaccine.

Time interval from the administration of the first dose (5×10^9)	Number of mice succumbed after the administration of 1 mg histamine				Agglutination titer
	prior to the second dose	24 hours after the second dose		5 days after the second dose	
		$0,5 \times 10^6$	5×10^9	$0,5 \times 10^6$ 5×10^9	
48 hours	3/15	6/10	6/10	8/12 10/12	0
5 days	12/12	11/12	8/10	6/10 7/10	16 +++
10 days	8/10	10/11	10/10	6/10 5/10	64 +++
15 days	6/20	5/10	7/15	2/12 2/12	128 +++
20 days	3/20	4/11	8/20	0/20 1/19	256 +++
25 days	0/20	1/12	2/20	0/20 0/20	512 +++

that by this time the revaccinated mice show evidence rather of a certain degree of increased resistance to histamine.

The control titration of antibodies in the serum of mice showed that antibodies detected by the agglutination reaction appear in the serum by the fifth day following the administration of vaccine in the dose of 5×10^9 . The peak of their occurrence is found to occur by about the 20th day following the vaccination; however, the level of these antibodies is found to be relatively high as early as by the 20th day, hence at a time by which the repeated injection of pertussis vaccine failed

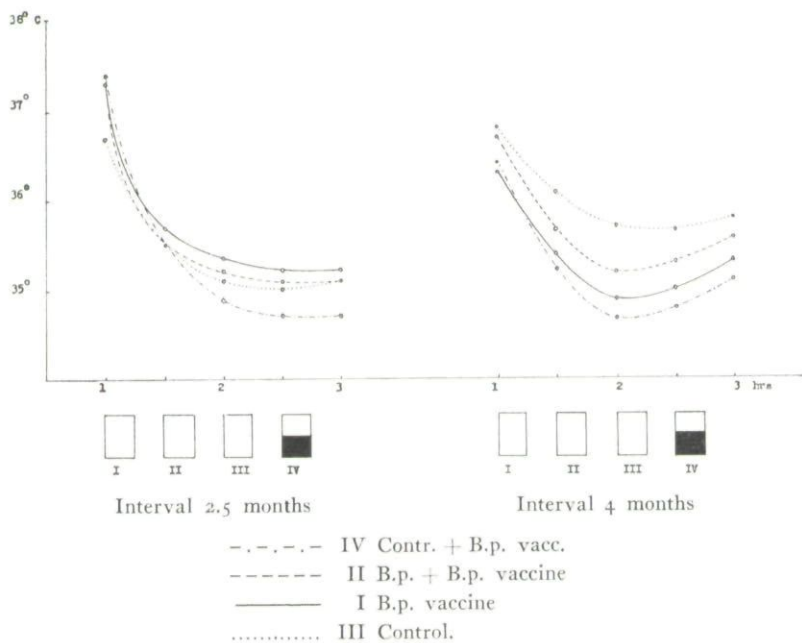


Fig. 1.

The effect of previous administration of pertussis vaccine on the sensitizing effect of a new dose of this vaccine (tested by the rectal temperature curve and the number of dead rats).

Note: The second injection of pertussis vaccine was administered at the interval of, respectively 2.5 and 4 months from the first injection.

to enhance the sensitizing effect of the first dose. The results confirm that in the immune organism further injection of pertussis vaccine cannot produce sensitization to histamine.

In order to demonstrate this property likewise for rats, another experiment was undertaken in which the animals received the second dose of pertussis vaccine after the time-elapse of 2.5 to 4 months. The results of this experiment are shown in Fig. 1. From the graph it is possible to deduce the following conclusions: The lethal effect of the administered histamine dose manifests itself only in the control group which received 7 days previously the sensitizing dose of 20×19^9 microbes; rats which had been sensitized 2.5 and 4 months

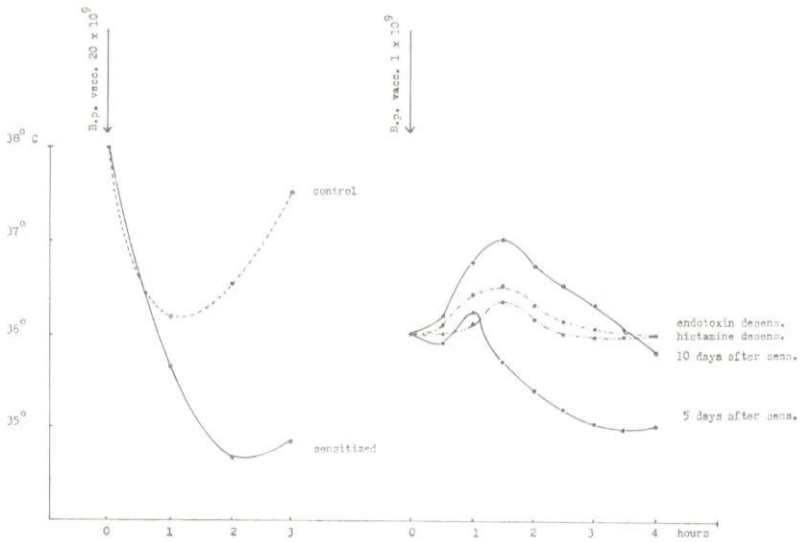


Fig. 2.

The course of rectal temperature in rats after administration of a low (1×10^9) or a high (20×10^9) pertussis vaccine dose; and an attempt to exert influence upon it by endotoxin and histamine desensitization.

previously did not respond by lethal shock, not even if they had been revaccinated or rather resensitized with the same dose 7 days prior to the histamine injection. If the course of shock is evaluated by the rectal temperature fall the differences are relatively smaller and not entirely uniform. Again, rats sensitized 7 days before the experiment exhibit the severest course of shock. By administering the second sensitizing dose 2.5 months after the first sensitizing dose no reactions are produced which would differ from the response found in the unsensitized control group. Four months after the first sensitization the unsensitized control group seems to be the least susceptible as compared to all of the remaining groups; however, the results are not uniformly demonstrable. Hence, in its sum this experiment confirmed the preceding results obtained on mice, namely the fact that the repeated administration of pertussis vaccine cannot produce in the immune organism any marked increase in the sensitivity to histamine.

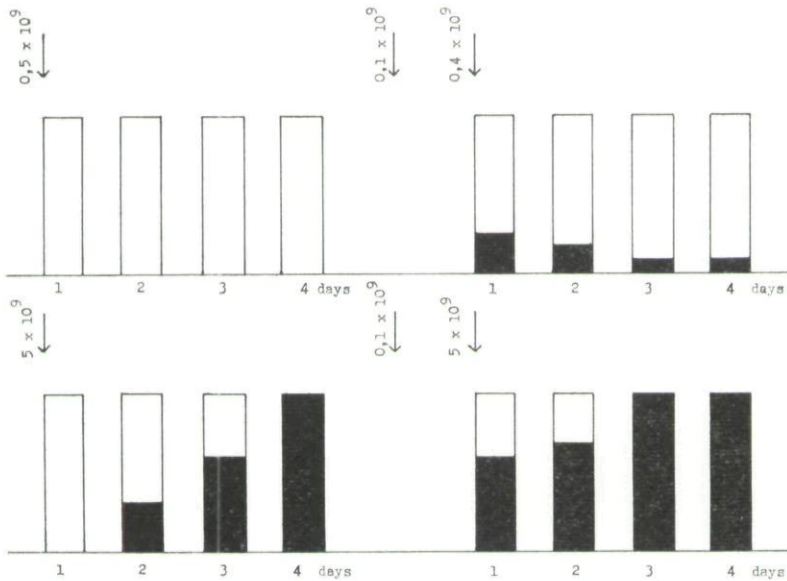


Fig. 3.

The effect of previous *B. pertussis* vaccine injection on the course of histamine shock in the mouse at various time intervals from the sensitization.

Note: The previous pertussis vaccine injection (0.1×10^9) was administered 5 day prior to the administration of the true sensitizing dose, i.e. 0.4×10^9 and 5×10^9 respectively.

Since it was found that the repeated administration of pertussis vaccine in the dose of 5×10^9 produced in mice a lethal shock whereas with the low dose remained free of this effect, an attempt was made to determine whether or not a perceptible response to the administration of a low dose, occurs at all. The experiment was undertaken on normal rats and on rats which had been previously sensitized with pertussis vaccine with a dose of 20×10^9 . At various time intervals after the primary sensitizing dose, the animals were given a relatively low dose (1×10^9) of pertussis vaccine and the response of the rat was tested by measurements of rectal temperature. Fig. 2 shows the temperature curve resulting from the administration of pertussis vaccine in the dose of, respectively,

20×10^9 and 1×10^9 to normal rats and rats sensitized with the 595/S₁ vaccine (in the dose of 20×10^9). From the graph it follows that the 20×10^9 dose produces in the rat a shock which is in the normal rat of a short duration; on the other hand in the sensitized rat this shock is profound and for the most part lethal. The administration of a low dose produces in the sensitized rat either fever (in animals in which the sensitization is of a longer duration) or—in animals in which the sensitization is of a shorter duration—shock with an initial mild rise in temperature. The effects of the low pertussis vaccine dose can be moderated by previous desensitization with endotoxin or histamine.

The change in temperature occurring after the administration of a low dose to sensitized rats proves the increased reactivity of their organism. It appeared therefore to be of interest to know whether or not changes occur also in the response of an organism which had been inoculated with a low dose only. The results of this experiment, summarized in Fig. 3, showed that previous administration of a low dose to a white mouse is able to alter the reactivity of this animal. A white mouse injected with pertussis vaccine will not respond with lowering resistance to histamine in less than 48 to 72 hours (see also Table I).

If, on the other hand, this dose is administered to a mouse which was previously sensitized with a low dose which by itself is not able to reduce the resistance to histamine, the symptoms of lowered resistance will appear as early as in 24 hours. On the other hand, as has been already shown, in normal (previously uninjected) mice the reactivity to histamine remains unaffected by the administration of pertussis vaccine, however, high its dose may be to 48 hours. We consider the principal difference in the chronological dynamics of the sensitization onset to be of extreme importance and significance.

In order to exclude in this case the influence of stress on the sensitizing properties, another experiment was undertaken in which the response to histamine was tested on mice inoculated with low pertussis vaccine doses. The results of this experiment, presented in Table II, demonstrate clearly that the administra-

TABLE III
Effect of stress on histamine sensitivity in white mice.

Dose of vaccine	Type of stress, produced on the 7th day following the basic vaccination	Number of animals succumbed after the administration of 1 mg histamine		
		24 hours	after stress	72 hours
100×10^6	Cold stress - 5 hours	1/10		0/10
100×10^6	B. paraptussis vaccine (dose 5×10^9)	3/10		1/10
100×10^6	Colcemide (3×0.05 mg) + B.p. vaccine (5×10^9)	6/15		—
100×10^6	B.p. vaccine (5×10^9)	18/30		17/20
100×10^6	Ø	0/10		0/10

tion of the second dose of persussis vaccine induces an increased susceptibility to histamine by another mechanism than mere stress action.

In the preceding experiments the altered reactivity was tested on mice receiving pertussis vaccine by the intraperitoneal route. An attempt was made to test this phenomenon on mice that received this vaccine by another route. First of all, tests were designed to determine the course of sensitization after various modes of pertussis vaccine administration and to compare it with that resulting from the intraperitoneal administration. As can be seen from Table IV, the administration into the paws produces a decrease in resistance to histamine at a somewhat later date but the maximum sensitivity occurs in both cases after

TABLE IV
Influence of the site of administration on the sensitizing properties of pertussis vaccine.

Mode of administration of the sensitizing pertussis vaccine dose (5×10^9)	Number of animals succumbed after the administration of 1 mg histamine at various time intervals					
	24 hours	48 hours	72 hours	96 hours	120 hours	240 hours
Intraperitoneally	0/10	5/10	5/10	4/10	10/10	4/10
Into paws	0/10	0/10	3/10	6/10	9/10	3/10
Intracerebrally	0/10	—	—	5/10	10/10	—
Intranasal route	—	0/12	—	—	0/14	—
Subcutaneously	—	1/10	—	—	6/10	—
Into thymus	—	7/11	—	—	8/10	—

TABLE V

Effect of various sites of administration of the repeated pertussis vaccine dose on histamine sensitization.

Site of administration of the primary dose 100×10^6	Site of administration of the repeated dose $5,000 \times 10^6$	Number of animals succumbed after the administration of 5 mg histamine 24 hours after the second pertussis vaccine injection given at various intervals					
		6 hours	24 hours	48 hours	7 days	14 days	21 days
Intraperitoneally	Intraperitoneally	—	13/29	5/10	10/10	7/10	5/10
Into the paw	Intraperitoneally	3/10	13/20	8/15	7/10	6/10	1/10
Into the paw	Into the opposite paw	0/10	7/20	11/18	5/10	5/10	2/10
Into the paw	Into the same paw	1/10	5/20	13/20	7/10	6/10	3/10

about the same time interval. The results show the intranasal route to be unsuitable. Besides the intraperitoneal administration, satisfactory results were also obtained by administering the vaccine intracerebrally, into the thymus and, to a lesser degree, subcutaneously.

In the actual experiment, tests were carried out to determine the response of the organism to low doses administered by the intraperitoneal route or into the paws with the use of various methods of revaccination. The results, presented in Table V, confirm that after the repeated administration of the sensitizing pertussis vaccine dose marked susceptibility to histamine develops as early as in 24 hours following the second dose while partial susceptibility develops even in as few as 6 hours. At this time (i.e. 24 hours after the second sensitizing dose) the susceptibility produced by the second dose proved to be higher on intraperitoneal administration than on subcutaneous administration; later on, the difference between these two groups becomes levelled and the sensitization course appears to correspond approximately to that found after the primary administration of pertussis vaccine. This result seems to be logically explainable on the basis of the antigen absorption rate. Between the sensitization by the intraperitoneal route and that produced by the administration into the paws, no significant difference are found, similarly as was shown in the preceding table.

DISCUSSION

The discussion of this study has to deal with two different problems. The significance of this study from the point of view of clinical medicine and pertussis vaccination on the one hand, and the attempt at interpretation and analysis of the observed phenomena on the other hand.

Pertussis vaccination is frequently accompanied by post-vaccination reactions and disagreeable complications. However, until recently these reactions were not related to the lowered resistance to histamine which occurs in mice and rats after the administration of pertussis vaccines prepared from strains in the 1. phase. On the other hand *Sanyal*, in 1961, reported on increased susceptibility to histamine in children after pertussis infection and vaccination. In order to determine whether or not the low doses are capable of inducing an altered reactivity in the organism an attempt was made to test the response of white mice to histamine after repeated and low doses of pertussis vaccine.

It is known that previous administration of a specific pertussis serum inhibits the rise of a new sensitization (*Maitland et al.*, 1955); similarly, in the hyperimmune organism it is impossible to potentiate by pertussis vaccination the development of anaphylactic shock (*Kopytovskaya*, 1958, *Chedid & Boyer*, 1960). In agreement with these authors our experiments demonstrated that repeated pertussis vaccination is not only free of any enhancing effect on the first sensitizing injection (provided the sensitizing effect is estimated the 5th day following the repeated injection), but that it essentially reduces the possibility of development of a new sensitization.

The administration of pertussis vaccine induces not only antibody formation but also changes in the reactivity of the whole organism. One of these manifestations is, for example, the lethal shock occurring after the repeated dose of pertussis vaccine. Though histamine is excreted during this shock (unpublished observation) the animal is not desensitized and becomes, contrarywise, more susceptible to histamine 24 hour

following the shock. Lowered resistance to histamine occurs temporarily likewise in animals revaccinated with a low dose which per se is not able to induce lowered resistance. This repeated low dose produces in the sensitized organism a characteristic response. Moderation of this reaction can be obtained by previous desensitization with histamine or endotoxin. The phenomenon involves in all probability a mixed reaction of the anaphylactic type, a reaction to the endotoxic constituent of the pertussis microbe and a reaction based on delayed type hypersensitivity. It was found (*Pekárek & Vrána, 1963*) that the lethal anaphylactic shock in pertussis sensitized rats can be inhibited by desensitization with histamine. As far as the favourable effect of endotoxin desensitization is concerned, this desensitization can exert a favourable effect even on other reactions than the resistance to endotoxin (*Stetson et al., 1958*).

If the organism is influenced by low doses which by themselves are not able to alter the reactivity of the organism to histamine, the organism develops—after the administration of the sensitizing dose proper—a rapid hypersensitivity. This rapid development of hypersensitivity indicates that the administration of low doses of pertussis vaccine produces in the organism some alteration. It is likely that this alteration involves immunological mechanism since it conforms typically to the known uniform regularity of the so-called secondary immunological response. Previous administration of non-specific vaccines (i.e. heated pertussis vaccine or endotoxin) had no effect on the acceleration of sensitivity. It is therefore possible that in organisms influenced by preceeding administration of a low dose of pertussis vaccine there is an accelerate deliberation of a special antigen fraction responsible for this type of sensitivity from the body of pertussis microbe. This component of *B. pertussis* microbe is then contained in various cells and organs of the sensitized organism and due to its presence causes the alteration of various functions.

If the immunological mechanism of the rise of sensitivity to histamine after the administration of pertussis vaccine is considered a striking parallelism appears between the mani-

festations of this sensitivity and the reactions of the delayed type hypersensitivity. First, sensitization with pertussis vaccine produces in experimental animals as well as in man "hypersensitivity of the delayed type" (Rowley *et al.*, 1959). Moreover, delayed type hypersensitivity was reported to be accompanied by a transient increase in susceptibility to the action of both endotoxin and histamine which occurs at time intervals corresponding approximately to the peaks of endotoxin sensitivity after sensitization with pertussis vaccine. An increased ability to form antibodies against a variety of other antigens was found to occur in the stage of delayed hypersensitivity as well as after the administration of pertussis vaccine (Kind, 1957, Gell & Benaceraff, 1960); in both cases the stage of susceptibility to the shock effect of histamine or endotoxin may be acted upon by the administration of corticosteroids (Kind, 1953). Finally, it is also known that the administration of a corresponding antiserum inhibits the development of the delayed type hypersensitivity to an antigen administered under conditions leading in other circumstances invariably to the development of the delayed type hypersensitivity, as it is the case, for example, with the Freund adjuvant. This interference of two primary immunological processes makes itself felt both in the hypersensitivity to protein antigens and tuberculin and in various transplantation reactions dependent on the hypersensitivity of the delayed type (Snell *et al.*, 1960). The parallelism of this phenomenon and the inhibitory effect of immune sera on the rise of sensitization to histamine after pertussis vaccination is striking.

Vejbora *et al.* (1961), in his work on the mechanism of increased susceptibility to endotoxin after BCG sensitization, presented experimental evidence to support the hypothesis that the increased susceptibility is in no way a product of the actual stage of hypersensitivity per se, being rather a result of reactions and events developing in the organism during the interaction of the hypersensitive cell with the antigen which persists in the organism. The same conclusions follow from the work of Box & Briggs (1961) who showed that the non-specific sus-

ceptibility to endotoxin subsided concurrently with the disappearance of the infective agent from the organism. If this hypothesis were correct it would be entirely logical why sensitization to histamine is produced only by a high and not by a low dose of pertussis vaccine, since in the latter case there are no such conditions for the persistence of the antigen in the organism. Of equal logical consequence is the finding that an increase in the susceptibility to histamine can be produced, after the administration of a low dose of pertussis vaccine, by the second dose of this vaccine; in the sense of the above hypothesis, the conditions for the interaction between the hypersensitive cell and the antigen would prove true. On the basis of the same principle it is possible to explain the early rise of susceptibility to histamine after the administration of the second dose of pertussis vaccine: specific hypersensitivity already exists and it need not develop progressively as in the case of the primary administration of the sensitizing vaccine dose.

According to this conception the manifestation of hypersensitivity to histamine produced by the administration of pertussis vaccine would be analogues of similar changes in susceptibility associated with delayed type hypersensitivity. As a prerequisite for the development of increased susceptibility it is possible to presume the formation of pharmacologically active substances induced by the immunological reaction between the hypersensitive cell and the antigen (*Johanovský, 1963*). The individual differences between delayed type hypersensitivity and the manifestations of sensitization produced by pertussis vaccine might be explained on the basis of different properties of individual antigens, different quantitative proportions and/or the participation of other mechanisms, e.g. anaphylactic hypersensitivity which occurs on pertussis vaccine sensitization.

We consider it necessary to lay particular stress on the fact that this interpretation represents only a working hypothesis and that no conclusions can be drawn from the parallel manifestations of these two types of reactions as to their analogous mechanism; however, partial participation of at least some elements of the delayed type hypersensitivity in the pathophy-

siology of pertussis sensitization seems to be extremely probable.

We call attention again to the significance of the observation from the point of view of the possible post-vaccination reactions. It is known that mice develop sensitization only after the administration of relatively high doses. On the other hand doses utilized for the prevention of whooping cough are much lower and this is why they were presumed to be free of the sensitizing effect. Our results have shown with a fairly convincingly that marked physiological influencing of the organism and an increase in its susceptibility to external noxious effects is brought about even under the conditions of repeated administration of relatively low doses of pertussis vaccine. This was the main reason why we concentrated during the development of methods for the preparation of pertussis vaccine, on technological procedures allowing elimination of the maximum possible amount of the histamine sensitizing factor without substantially reducing the protective power of the vaccine (*Pekárek et al.*, 1961). In fact, in experiments the Czechoslovak pertussis vaccine is free of the sensitizing effect; on clinical administration it produces reactions in a much lower percentage and of a minor significance as compared to foreign vaccines, and exhibits at the same time a high epidemiological effect (*Pekárek & Stejskal*, 1962).

SUMMARY

The response of mice and rats to low doses of pertussis vaccine was investigated. It was found that repeated sensitizing doses produce in the organism thus influenced a lethal shock. Repeated injection of a low dose produces a febrile or shock response. The enhancement of the sensitizing properties after the repeated dose can be proved only within 24 hours following the administration of this vaccine. In most cases further increase in sensitization does not occur this being probably brought about by the increased content of antibodies.

Previous influencing of the organism by a low dose which

by itself is not able to produce sensitization, accelerates the development of sensitization after the administration of the actual sensitizing dose. This observation proves that the process of sensitization may be influenced even by low doses of pertussis vaccine.

Furthermore, the probability of the immunological mechanism of this phenomenon, and the close relation of the pertussis vaccine-induced reactions to the manifestations of the delayed type hypersensitivity were discussed.

Attention was drawn to the clinical significance of the sensitization induced by repeated doses of pertussis vaccine, and stress was laid on the importance of preparing pertussis vaccines free of sensitizing properties.

REFERENCES

- Box, E. D. & Briggs, N. T.: Endotoxin susceptibility and delayed hypersensitivity in experimental histoplasmosis. *J. Immunol.* 87, 485, 1961.
- Chedid, L. & Boyer, F.: Nouvelles recherches sur l'action histaminsensibilisant de *H. pertussis* (phase I). *Annales de l'Institut Pasteur* 99, 314, 1960.
- Gell, P. G. H. & Benacerraf, B.: The significance of the stage of delayed hypersensitivity to antibody formation. *Mechanisms of Antibody Formation*, Prague 1960, page 152.
- Halpern, B. N. & Wood, D. R.: The action of Promethazine (phenergan) in protecting mice against death due to histamine. *Brit. J. Pharmacol.* 5, 510, 1950.
- Johanovský, J.: Experimental basis for the Hypothesis of the Existence of a specific Mediator in Hypersensitivity Reactions of the Delayed Type. *Čas. lék. čes. CII*, 364, 1963 (czech.)
- Kind, L. S.: Inhibition of histamine death in pertussis-inoculated mice by cortisone and necantergan. *J. Allergy* 24, 52, 1953.
- Kind, L. S.: Relationships of the anaphylaxis sensitizing and adjuvant properties of *Hemophilus pertussis* vaccine. *J. Immunol.* 79, 238, 1957.
- Kind, L. S.: The altered reactivity of mice after inoculation with *Bordetella pertussis* vaccine. *Bact. Rev.* 22, 173, 1958.
- Kopylovskaya, L. P.: Kizuezeniju sensibilizirujushchevo dejstviya kokljushnykh vakcin. *Jezhegodnik III*, 293, 1958.
- Maitland, H. G., Kohn, R. & Mac Donald, A. D.: The histamine sensitizing property of *Hemophilus pertussis*. *J. Hyg.* 83, 196, 1955.
- Parfentjev, I. A.: Increase susceptibility to *Shigella* endotoxin and to histamine after immunization to *H. Pertussis*. *Yale J. Biol. Med.* 27, 46, 1954.

- Parfentjev, I. A.*: A hypersensitivity phenomenon in animals bearing tumors. Proc. Am. Assoc. Cancer Research 2, 38, 1955.
- Parfentjev, I. A.*: Anaphylaxis and histamine shock in mice. Proc. Soc. exp. Biol. Med. 89, 297, 1955.
- Parfentjev, I. A. & Goodline, M. A.*: Histamine shock in mice sensitized with *Hemophilus pertussis* vaccine. J. Pharmacol. Exp. Therap. 22, 411, 1948.
- Pekárek, J., Stejskal, A. & Kvapilová, M.*: Nový způsob přípravy pertusové vakcíny (in Czech). Čs. epid., microbiol., immunol. X, 314, 1961.
- Pekárek, J. & Stejskal, A.*: Preparation of non-reactive and non-sensitizing vaccine. Pertussis conference Prague 1962.
- Pekárek, J. & Vrána, M.*: Effect of Pertussis Vaccination on Anaphylactic Shock in Mice and Rats. J. Hyg., Epid., Microbiol., Immunol. VII, 28, 1963.
- Pittman, M.*: Sensitivity of mice to histamine during respiratory infection by *Hemophilus pertussis*. Proc. Soc. exp. Biol. Med. 77, 70, 1951.
- Rowley, D. A., Chutkow, J. & Attig, Ch.*: Severe active cutaneous hypersensitivity in the rat produced by *Hemophilus pertussis* vaccine. J. Exp. Med. 110, 751, 1959.
- Sanyal, R. K.*: Histamine sensitivity in man. Internat. Arch. Allergy and Appl. Immunol. 18, 197, 1961.
- Snell, G. D., Winn, H. J., Stimppling, J. H. & Parker, S. J.*: Depression by antibody of the immune response to haemografts and its role in immunological enhancement. J. Exp. Med. 112, 293, 1960.
- Stetson, Ch. Jr., Schlossmann, S. & Benacerraf, B.*: Endotoxin-like effects of old tuberculin. Fed. Proc. 17, 536, 1958.
- Vejbora, O., Johánovský, J. & Vrána, M.*: Dynamics of changes in specific hypersensitivity and of non-specific increase in sensitivity to endotoxin in BCG-sensitized mice. Folia microbiol. 6, 370, 1961.

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