



REPORT ON AN INFORMAL CONSULTATION ON
 IMMUNIZATION AGAINST WHOOPING-COUGH

Rijks Instituut voor de Volksgezondheid
 Bilthoven, Netherlands

9-11 December 1974



CONTENTS

	<u>Page</u>
List of participants	2
1. Introduction	3
2. Whooping-cough in the world	3
3. Vaccines against pertussis	4
4. Untoward reactions to vaccination	5
5. Immunity	7
6. Conclusions and recommendations	7
Annex I. Toxic factors of <u>B. pertussis</u>	10
Annex II. The different types of reaction after DPT-Polio vaccination and their frequency in the Netherlands	16
Annex III. Pharmacological phenomena occurring after pertussis vaccination	26

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted or quoted without the agreement of the World Health Organization. Authors alone are responsible for views expressed in signed articles.

Ce document ne constitue pas une publication. Il ne doit faire l'objet d'aucun compte rendu ou résumé ni d'aucune citation sans l'autorisation de l'Organisation Mondiale de la Santé. Les opinions exprimées dans les articles signés n'engagent que leurs auteurs.

LIST OF PARTICIPANTS

Dr H. C. Bartlema, Medisch-Biologisch Laboratorium TNO, Lange Kleiweg 139, Rijswijk, Netherlands

Dr B. Burianova-Vysoka, Chief, Department of Epidemiology, Medical Faculty of Hygiene, Charles University, Srobarova 48, Czechoslovakia

Professor J. A. Dudgeon, The Hospital for Sick Children, Great Ormond Street, London WC1N 3JH, United Kingdom

Dr G. Edsall, Department of Microbiology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, United Kingdom (Rapporteur)

Dr D. S. Freestone, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent, United Kingdom

Dr A. H. Griffith, Head, Department of Clinical Immunology, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent, United Kingdom

Professor Walter Hennessen, Behringwerke A.G., Marburg 355, Federal Republic of Germany

Dr N. W. Preston, University of Manchester, Manchester, United Kingdom

Dr E. H. Relyveld, Institut Pasteur, 92380 Garches, France

Dr M. Tiru, Statens Bakteriologiska Laboratorium, 10521 Stockholm, Sweden

Professor D. de Wied, Rudolf Magnus Instituut voor Farmacologie, Vondellaan 6, Utrecht, Netherlands

Professor M. S. Zakharova, Chief, Laboratory of Respiratory Infections, Gamaleya Institute of Epidemiology and Microbiology, Gamaleya Street 18, Moscow 123098, Union of Soviet Socialist Republics

Staff of the Rijks Instituut voor de Volksgezondheid, Bilthoven, Netherlands

Dr H. Cohen, Director (Chairman)

Dr Ch. A. Hannik, Paediatrician

Dr J. Nagel, Chief, Immunochemistry Unit

Dr J. D. van Ramshorst, Chief, Department of Biological Standards

Dr P. A. van Hemert, Chief, Laboratory for Vaccine Production

WHO Secretariat

Dr B. Bytchenko, Bacterial Diseases, WHO, Geneva, Switzerland (Secretary)

Dr W. Chas. Cockburn, Director, Communicable Diseases, WHO, Geneva, Switzerland

Dr B. Cvjetanovic, Chief Medical Officer, Bacterial Diseases, WHO, Geneva, Switzerland

1. INTRODUCTION

Dr Cockburn opened the consultation on behalf of WHO and outlined its purpose: to review progress as regards the methods of preparation and testing of whooping-cough vaccine and studies directed towards the development of new, more effective and less toxic antigens; and to propose further research aimed at improving immunization programmes. Whooping-cough was still a severe disease; in the developed countries it had been successfully combated through immunization but in the developing countries it was still a cause of high morbidity and mortality among infants due to a lack of efficient immunization programmes. Immunization with DPT had sometimes given unsatisfactory results due to low potency of the pertussis component. Moreover, the toxic properties of pertussis antigens had been a cause of concern, particularly in those developed countries where, due to immunization, the disease had ceased to be a public health problem while reactions, although rare, had continued to occur. Further research leading to the development of more potent and less reactogenic pertussis vaccines was needed to achieve maximum success in the control of pertussis.

2. WHOOPING-COUGH IN THE WORLD

WHO reports on the world health situation, published since 1954, and based on information provided by Member States, indicated that pertussis continued to be a worldwide disease.

According to data supplied by 81 countries, about 350 000 children suffered from pertussis every year. The true number of cases was considerably higher as there was much under-reporting, especially in the developing countries. A cyclic rise in the incidence of pertussis was commonly observed every three to four years.

Before the era of immunization against pertussis it was found that 60-90% of the total adult population in certain countries had had pertussis at some time during their lives. This was probably still true for the majority of developing countries where little progress had been made in the prevention of the disease. An increase in morbidity had recently been noticed in Africa (Chad, Congo, Gabon, Senegal, etc.). In tropical areas, pertussis rivalled measles in importance and severity among young children, many of whom subsequently developed kwashiorkor or marasmus. As case-fatality rates due to pertussis in developing countries varied from 4% to 15% in infants, the total number of deaths from this disease was likely to be several millions per year.

The improvement of socioeconomic conditions in the developed countries, accompanied by the development of curative and preventive medical services had resulted in a twenty- to fifty-fold or even greater reduction in morbidity due to pertussis in the last two decades. Programmes of active immunization played an important role in this change.

In the USSR, the incidence of the disease dropped within 20 years from 400/100 000 to 12/100 000 population, and in Massachusetts (United States of America) the number of cases of pertussis fell from over 10 000 in 1930 to 27 in 1973.

In an immunized population the rapid decline of pertussis was generally characterized by less intensive outbreaks, by the disappearance of disease from certain areas, and by a change in the clinical pattern of the disease from severe to mild forms.

The majority of the developed countries were now experiencing disease caused by serotype 1,3. The epidemiological importance of B. pertussis (1,2,3; 1,2; 1,3) serotypes had not yet been fully established. So far there was no unequivocal evidence that the B. pertussis vaccines currently used for active immunization were not effective, provided they were produced and standardized in accordance with the WHO requirements.

The problem of parapertussis needed further attention and investigation. Outbreaks of parapertussis had been reported in Czechoslovakia, USSR, and some other countries more frequently than elsewhere. According to the WHO Serum Reference Bank, sera collected from the populations of various countries often contained parapertussis antibodies.

Reports from different countries showed that pertussis might occur in vaccinated children. This might be due to:

- (1) insufficient level of population immunity due to the use of low-potency vaccines or insufficient coverage;
- (2) the use of vaccines that did not match the B. pertussis serotypes isolated from the population;
- (3) a relative increase in the occurrence of infections due to B. parapertussis.

Further progress in the control of pertussis might be facilitated by studies on:

- (1) morbidity and mortality due to this disease in developing countries;
- (2) the needs of local medical services for the effective planning and implementation of immunization programmes;
- (3) the state of immunity of the population to pertussis and parapertussis, particularly in places where the disease affected immunized children;
- (4) the side-effects and complications caused by vaccines with a pertussis component;
- (5) the effectiveness of immunization programmes in terms of lives saved and economic gain or loss.

The effects of immunization programmes against pertussis and their cost-benefit aspects had not been sufficiently studied. However, Dr Zakharova said that planned active immunization against pertussis in the USSR since 1961 had saved about 8 000 000 children from the disease and preserved as many as 800 000 of them from neurological complications.

3. VACCINES AGAINST PERTUSSIS

The inclusion of strains of the three major agglutinogenic types in vaccines was discussed. It was pointed out that a variety of mutations could occur in the pertussis serotypes, e.g., 1,2,3 to 1,2 or 1,3; 1,2 to 1; 1 to 1,2 or 3; or the reverse of any of these. It was noted that mutations occurred in vivo - in children and in experiments on marmosets - and evidence was cited to support the conclusion that in poorly vaccinated children or animals such mutations or shifts tended to occur in such a way as to "evade" the pattern of immunity established by the vaccine.

Some participants expressed the opinion that superinfection might play an important role and that many infections might be mixed in serotype. It was noted that some evidence did not support the concept that serotypes were of major importance in protection. However, since it was found that some strains were poor producers of factor 3 some manufacturers had added 1,3 strain to their vaccines, thereby presumably increasing their agglutinogenic properties. Further investigations were considered desirable.

Progress in preparing and studying reference typing reagents at the WHO Collaborating Centre for Reference and Research on B. pertussis in Moscow was reported. The Centre had prepared type-specific sera which were being investigated by laboratories in other countries.

It was noted that a very important factor in community protection was the percentage of vaccine acceptance. When this was above 60% a decrease of the disease was observed, and when it was above 90% the disease virtually disappeared.

The probable significance of the high molecular weight and low molecular weight mouse-protective antigens (MPA) was reviewed. The low molecular weight preparations contained (in addition to MPA) histamine-sensitizing factor (HSF), lymphocyte-promoting factor (LPF) and haemagglutinin (HA). As some of these factors were found in the supernatants of B. pertussis cultures, suspensions of bacterial cells for vaccine production should be centrifuged and washed. Thermolabile toxin should be destroyed either by heating the bulk suspension, or by storing in merthiolate for long periods at low temperatures. In any case, the presence of traces of thermolabile toxins should be tested for by the mouse toxicity test in order to exclude them from the vaccine. There was a need for the preparation of high molecular weight MPA that was relatively free from the other factors.

Two methods of preparing cell fragment suspensions were described, by freeze-thawing (in Sweden), and by ultrasonic disintegration of the cell wall followed by ether extraction (in the USSR). The products obtained by the latter method showed high mouse-protective activity and low HSF, LPF, and other toxic factors. They were stable and showed fewer precipitation lines in agar gel diffusion tests. Preliminary studies in a small number of children showed these preparations to be less reactogenic than whole-cell vaccines.

Further investigations of these procedures should be undertaken. Special attention should be paid to the behaviour of fractions in the mouse-protection test, especially since such tests showed an unusually low dose-response slope with the alcohol-extracted freeze-thawed preparation.

The nine or 10 known toxic components of the pertussis bacteria were surveyed (see Annex I) together with some preliminary results on the preparation of LPF and the determination of endotoxin by the Limulus-lysate test. The LPF test could probably replace the more inaccurate HSF test.

The differences in toxicity for mice between centrifuged and acid-precipitated vaccines were described, the latter being generally more toxic. Testing of the vaccines in mice at eightfold concentration levels by observation of the lethal effect gave more information than the usual mouse weight-gain test. The effect was also observed when strains from different sources were used.

The observations that the potency of pertussis vaccines in the intracerebral mouse test decreased after adsorption onto aluminium hydroxide were confirmed. The same phenomenon was observed with calcium phosphate. It was shown the the adsorption of pertussis cells onto these adsorbents was more complete than onto aluminium phosphate. Aluminium phosphate, however, did not reduce the potency, the adsorption being less. On the other hand, histological studies showed that all adsorbed pertussis vaccines would provoke more pronounced reactions at the injection sites than the adjuvant by itself.

Considerable discussion revolved around the usefulness of the mouse-protection test. The consensus was that, though it could not be relied upon entirely to give an accurate indication of the efficacy of any given vaccine in man, nevertheless it had to date not yielded any known data that were seriously in error.

4. UNTOWARD REACTIONS TO VACCINATION

Since the introduction of DPT-Poliovaccine in the Netherlands in 1962, 47 cases of prostration,¹ 39 cases of convulsions, and 47 cases of persistent screaming had been observed.

¹ Described by some authors as a shock reaction (Annex II).

The early signs of prostration in babies were agitation and restlessness. The child became less and less responsive. The skin was pale, moist and cold. Newborns in a state of prostration appeared pale and grey. Respiratory distress (dyspnoea) and cyanosis were commonly observed in such patients, who eventually recovered without any sequel.

The incidence of prostration that was considered to be vaccine-induced was estimated to be about one in 3500 vaccinated infants. The incidence of convulsions was estimated to be about one in 2150 vaccinated infants (see Annex II). However, a part of the convulsions, although induced by vaccination, could often be attributed to neurological predisposition.

At least four cases with severe neurological symptoms after DPT-Polio vaccination were related to a primary infection with viruses (Herpes simplex, Coxsackie B5; Echo 25). The incidence of death after vaccination was estimated to be less than one per million vaccinated infants.

It was suggested that all the different reactions, including the minor and major reactions, might be considered to be one spectrum varying in degree, dependent on a predisposition in the child itself. Others felt that these various reactions might be the result of several different causes.

Studies of the pharmacological phenomena induced by pertussis vaccination in mice and rats showed that vaccination induced a β -adrenergic stimulation manifested by a marked elevation of the insulin level, although there were different symptoms similar to those observed after β -adrenergic blockade (see Annex III).

In evaluating reactions to vaccines, difficulties were encountered in deciding whether serious medical episodes occurring within days or weeks of vaccination were directly or indirectly attributable to administration or were unassociated with it. It was known that neurological conditions of unknown etiology occurred without warning in hitherto healthy infants at ages when vaccine normally was given. These included:

Sudden unexpected deaths - the incidence in children aged 2-6 months according to a number of surveys was 1.1:1 000 000 per day, and in children 6-12 months 4:1 000 000 per day.

First convulsions - in children aged 6-18 months they occurred at the rate of one to two per 100 000 children per day.

It should not be concluded, however, that all post-vaccination reactions were unconnected with vaccination. Vaccine-attributable reactions should show a time-related clustering of reactions additional to the "constant background level".

Since its inception in 1964, the Adverse Reaction Sub-Committee of the British Committee on Safety of Drugs (now the Committee on Safety of Medicines) had recorded untoward clinical events after the administration of drugs and vaccines. It was recognized that the reporting of reactions was incomplete although it seemed likely to be more complete for more serious reactions. From 1964 to March 1974, 892 reports of adverse reactions to DPT vaccines had been received, of which 21 were fatal.

A hospital discharge study in nine health regions of the United Kingdom in 1972 disclosed 35 hospital admissions following inoculation, at least five of which might have been severe. Eight encephalopathies and five deaths associated with DPT vaccination were reported to one producer during the distribution of 13 000 000 doses of vaccine. Several deaths when closely investigated had proved due to unrelated causes (such as tuberculous meningitis, Reye's syndrome). Thus the reporting of reactions did not necessarily imply a cause-and-effect relationship and often when additional information had been available other pathological conditions had been implicated.

In the United Kingdom, recent publicity had suggested that there might be a considerable number of serious persistent neurological effects induced by pertussis-containing vaccines. Although these suggestions had not been substantiated, it was understood that the use of DPT vaccine had declined sharply, and indeed all routine immunizations in infants, including measles vaccinations, had decreased.

In Estonia, USSR, no severe reactions had been observed after about 307 000 DPT injections during six years; the vaccine was produced on solid medium, and it contained 10 International Opacity Units. Moreover, the first vaccination was given at 5-6 months. Major reactions were not a problem in the USSR, Czechoslovakia or France. During the last five years no severe reactions had been observed in Sweden, where a reliable system of reaction reporting had been established and where 80 000 children were vaccinated yearly. In the USSR there had been rare and not necessarily vaccine-associated deaths - one per 10 000 000 DPT inoculations.

There was need for a uniform system of recording reactions and comparing their incidence, nature, and severity with similar conditions arising in non-vaccinated children of similar age-groups.

There was general agreement that vaccine-associated reactions were largely, if not entirely, attributable to the pertussis component in triple and quadruple vaccine. It was reported that some countries had recently reduced the number of international units of antigen in the vaccine; however, these vaccines still met the WHO requirements for potency.

5. IMMUNITY

Studies to evaluate the relative significance of humoral and cell-mediated immunity in protection and recovery from B. pertussis infection were reported.

In initial experiments, active and passive protection tests were carried out in X-irradiated and partly or totally immunologically restored mice, using standard immunization and challenge procedures. It appeared that the active protection in the mouse test, whether based on humoral or on cell-mediated immunity, might be thymus-dependent; the agglutination titre in the mice did not show any correlation with the rate of protection. The experiments had not so far yielded any conclusive evidence as to whether cell-mediated or humoral immunity alone could afford protection in the mouse test.

It was agreed that to date neither the studies in mice nor those in monkeys had yet determined whether immunity to pertussis was primarily humoral, local-antibody-mediated, cell-mediated, or a combination of some or all of these mechanisms, and that further investigations were necessary.

6. CONCLUSIONS AND RECOMMENDATIONS

Pertussis was a worldwide clinically serious disease with high mortality and complication rates. It could most effectively be controlled by vaccination. A twenty- to fiftyfold or greater decrease in incidence had been observed in countries where a high percentage of susceptible age-groups were vaccinated. Although pertussis in the vaccinated was usually mild, there were no indications that the disease had become milder in unvaccinated children and it was still difficult and expensive to treat.

1. In the light of recent knowledge and the evident benefits of vaccination, wide vaccination coverage of susceptible age-groups both in developed and developing countries should be maintained. Because of the importance of the disease in the developing world, the group strongly recommended WHO to pursue energetically its Expanded Programme of Immunization in the countries concerned.

2. Before starting an immunization programme the epidemiological situation should be studied by making clinical, bacteriological and serological examinations. It was emphasized that fluorescent antibody techniques might facilitate the diagnosis of the disease. In an immunized population mild and atypical cases clinically resembling pertussis were observed. They might be due to B. pertussis or to such organisms as B. parapertussis and H. influenzae type B, or perhaps certain adenoviruses.

3. There were different clinical definitions of whooping-cough ranging from mild paroxymal cough to the classical symptoms of whooping and vomiting. Clinical severity should be assessed by the number of paroxysms at the height of the illness, the duration of the paroxymal cough (by weeks), and the presence or absence of typical whooping and vomiting.

4. It had been shown that there were three major agglutinogenic patterns of B. pertussis (1,2,3; 1,2; and 1,3) which might be unstable and might interchange either by mutation or selection. Mixtures of these serotypes could be found in human infections. Pure type 1 strains were seldom encountered. Similar observations had been made in experiments on marmosets and there were indications in this species that immunity might be type-specific. The practical importance in vaccine production of the differences in agglutinogenic structure was not clear but there should be suitable strains of the major serotypes in the vaccines. Many countries had been successful in making potent vaccines with their own strains, so at present there was no need to make strains available from a central source. However, it was strongly recommended that WHO continue to make available sera for typing to enable producers to control the agglutinogenic pattern of their strains and to facilitate identification of the isolated strains. It was also advisable to determine in animals and man the agglutinogenic properties of the vaccines.

5. For the calibration of vaccines by the mouse-protection test each country should have its national reference preparation. The test might give unreliable results, one cause of which (among others) might be variations in the quality of national reference preparations. Therefore, the national reference preparation should be checked annually against the international standard preparation. The international standard preparation, which was many years old, should be restudied.

6. Much was now known of the nature of the factors toxic for mice and other animals. At least some of them seemed to be closely associated, and simple methods had been developed to measure such factors as LPF and endotoxin quantitatively. It was recommended that collaborative studies be set up to measure these factors in vaccines containing B. pertussis antigens of different origins. Reference preparations and standardized techniques for the measurement of the factors should be worked out.

7. Encouraging results had been reports from the USSR with a cell-fragment pertussis vaccine that met the WHO requirements for potency and absence of toxicity. It was recommended that this vaccine be made available for tests in other laboratories and that the pilot studies in infants already commenced in the USSR be continued. Studies on the purified antigen in other laboratories should, of course, also be encouraged.

8. A great deal of attention had recently been given to the question of untoward reactions, in particular to prostration, convulsions, and persistent screaming. The prostration state was rarely observed in some children who, within several hours after vaccination, suddenly turned ashen, and became cold and still. From an intensive national study it seemed that symptoms of prostration appeared in 1/3500, and convulsions in 1/2500 vaccinated infants. The state of prostration disappeared in a few hours and part of the convulsions were of the febrile type.

In the same careful study over a period of 12 years in which 3 000 000 children were vaccinated, six more serious time-associated reactions were reported. In two of these, herpes virus infection might have been etiologically responsible. One other recovered

completely, one had a permanent mild hemiparesis, and two were rapidly fatal. In studies in other countries the rates were apparently similar or much lower.

It was recommended that collaborative studies on untoward reactions be established on uniform lines in a number of countries and that at the same time the properties of the vaccines used be studied.

9. From reports from various countries it was probable that in families with a history of neurological disorders, neurological complications including convulsions were observed more frequently after vaccination; care should be taken not to vaccinate children from such families.

10. Studies of the pharmacological phenomena occurring after pertussis vaccination should be continued in animals and should also be initiated in children.

11. The data presented to the group indicated a high level of protection in well vaccinated communities. Authenticated reactions endangering the health or life of the children vaccinated were extremely rare, probably less than one per million. It was noted with concern that recent publicity unsupported by scientific evidence on reaction rates had had a serious effect on the acceptance of vaccination in at least one country. There was no evidence that the disease was less severe now than it had been in the past. A fall-off in vaccination would lead to widespread outbreaks of the disease as experience in several countries had already shown.

12. The nature of the immunity after pertussis vaccination was still obscure. It was recommended that studies be carried out on the cellular and humoral mechanism of immunity after vaccination and on the role of the different biologically active cell components. The development of tests to define these mechanisms would be particularly important in the screening of purified vaccines.

ANNEX I

TOXIC FACTORS OF B. PERTUSSIS

by

J. Nagel

Many properties of B. pertussis have been described in the literature. Besides the favourable protective activity and the innocuous agglutinin, various more or less toxic factors are known (Munoz, 1963; Munoz & Bergman, 1968). They comprise:

- (1) the heat-labile, dermonecrotic toxin
- (2) the endotoxin or lipopolysaccharide
- (3) the haemagglutinin
- (4) the histamine-sensitizing factor (HSF)
- (5) the late-appearing toxin as described by Kurokawa and others
- (6) the leukocytosis or lymphocytosis-promoting factor (LPF)
- (7) the adjuvant activity, in particular that stimulating the production of homocytotropic (IgE-like) antibodies
- (8) the active and passive anaphylaxis-promoting activity
- (9) the shock-promoting activity
- (10) the factor responsible for various metabolic changes after the injection of vaccine.

In this compilation, the heat-labile toxin and endotoxin clearly stand apart. In spite of many investigations, few people claim to have separated these factors. For that reason, the common opinion is that they are associated with the same molecular entity.

From the point of view of vaccine production, the heat-labile toxin causes no problem. It is destroyed by heating the vaccine to 56°C.

The endotoxin, extracted from the bacteria by the heat-phenol method of Westphal et al. (1952), does not seem to be very toxic. However, a more lethal endotoxin has been described by Kitagawa et al. (1961). According to Mota et al. (1974), endotoxin may be responsible for the enhancement of IgE-like antibody production in guinea-pigs. Recently, a very sensitive in vitro test has been developed. It is based on the gelation of a blood-cell lysate of the horseshoe crab (Limulus lysate). In this test, picogram quantities of endotoxin can be detected (Thye Yin et al., 1972).

Very little is known about the haemagglutinin and its possible toxicity. The available publications relate only to its isolation.

The adjuvant activity, apart from that caused by the endotoxin, has been studied, especially in relation to the production of homocytotropic, i.e. anaphylaxis-promoting, antibodies in mice and rats (Munoz, 1964). There seems to be a close relationship between this activity and the HSF. The same is true of the activity provoking hypersensitivity reactions against various substances such as serotonin, peptone, endotoxin, etc. (Levine & Pieroni, 1966).

Annex I

The HSF has been studied extensively, both by immunologists and pharmacologists (Lehrer et al., 1974). Many investigations have been performed to elucidate its mode of action in experimental animals. Although it is thought that the adrenals may be involved, its exact mode of action remains obscure. But as it is closely related to the anaphylaxis and shock-promoting activity and provokes many physiological changes in animals, its toxic nature stands beyond all doubt. However, there exists also a close relationship with the mouse-protective antigen and only a few investigators have claimed to have separated both activities (on an analytical scale). A Swedish group claims to be able to produce an HSF-free vaccine on production scale.

The leukocytosis-promoting factor, which is most probably identical to the late-appearing toxicity factor, has recently attracted attention (Sato et al., 1972, 1973, 1974). This factor, which is mainly excreted into the culture supernatant, is of little importance for vaccine production provided centrifuged cells are used. But it is known from investigations in our Institute that the LPF will be precipitated together with the pertussis cells when the pH of the culture is lowered to a value of 3.5-4, as is done when the cells are harvested by acid precipitation (van Hemert, 1969). Vaccines containing acid-precipitated cells give toxic reactions (Cohen et al., 1969). The mouse weight-gain test indicates the presence of this toxin by weight decrease and death of the mice later than the third day after injection.

Sato (Sato et al., 1974) recently presented an interesting hypothesis about the relationship between the mouse-protective antigen, the HSF, the LPF, and the haemagglutinin. From his investigations of the properties of the LPF isolated from culture supernatants he drew the following conclusions.

Bordetella pertussis produces two types of protective antigen: first, an HSF-free, LPF-free, non-toxic protective antigen (Sato, 1970; Zakharova et al., 1970) which is located in the cell wall, and, second, a much smaller molecule showing histamine-sensitizing, leukocytosis-promoting, and haemagglutinin activity. This conclusion was based on the following results: (1) purified LPF showed, after mild formalin detoxification, a pronounced protective activity; (2) an antiserum prepared in rabbits against the LPF protected mice passively; and (3) after absorption of the anti-LPF antiserum with purified LPF, all protective antibodies were absorbed from the serum. Absorption of an anti-whole-cell antiserum in the same way did not diminish its protective activity.

Electromicrographs of the HSF-free mouse-protective antigen, isolated from bacteria, showed that the 22 S-molecule consists of cylindrical molecules, each composed of ring-shaped substructures. On the other hand, the toxic protective antigen, the purified LPF, consists of very small filaments.

This filamentous material could be demonstrated by Morse & Morse (1970) to be present on the bacteria during the first 48 hours of cultivation. After that time, especially after four days, very few filaments remained attached to the cells. In phase-IV bacteria these filaments could not be identified with certainty.

The existence of two types of mouse-protective antigen is a very useful hypothesis. As the toxic protective antigen has a strong affinity for the cell walls of erythrocytes, this might explain the rather high toxicity of Pillemer's S.P.A.-vaccine in children (Medical Research Council, 1959). Furthermore, Pillemer was using cells harvested after a 40-hour cultivation period. Such young cells would have the filamentous material still attached to the cell wall. Sato could demonstrate them on the stromata of erythrocytes after absorption of a sonic extract by the Pillemer method.

Annex I

Further support is given to Sato's theory by Clausen et al. (1968). Their highly purified HSF preparation caused a pronounced lymphocytosis. They showed, furthermore, that after the injection of anti-mouse leukocytic serum the leukocytosis was markedly reduced while the histamine sensitivity was not affected. So, although both phenomena may be provoked by the same molecular entity, they may be acting independently of each other. The same holds true for other phenomena such as hypoglycaemia and adjuvant activity in relation to the production of homocytotropic antibodies.

From the above it becomes clear that the close relationship existing between the mouse-protective antigen and the factor that provokes various adverse reactions creates serious problems in the production of a non-toxic vaccine.

However, the mechanism of these toxicity reactions in experimental animals is not necessarily the same as that of the untoward reactions in children, for some marked differences exist between the two models. In the first, animals (mice and rats) in adolescence are used. Furthermore, the reactions are provoked by the injection of bacterial suspensions or soluble products. They often are maximal after three to four days. The second model consists of young infants who mostly receive a vaccine containing an adsorbent. Moreover, the symptoms of toxicity are manifest within a few hours after vaccination.

Whatever the toxic agents causing the adverse vaccination reactions may be, it will be important to have laboratory procedures available for the standardization of such agents. For that reason an effort is being made to isolate purified HSF/LPF and purified endotoxin preparations. The experiments carried out until now comprise the following subjects.

1. Biological test for LPF

Preliminary experiments have been performed to test the homogeneity and sensitivity of various mouse strains in their response to the leukocytosis-promoting factor. Table 1 summarizes the results of experiments in which groups of 10 mice of each strain available received intravenous injections of 0.1 ml of extracts of culture supernatants of B. pertussis. After three days blood was taken from the tail vein. The leukocytes were counted in a haemocytometer.

TABLE 1. COMPARISON OF THE HOMOGENEITY AND SENSITIVITY OF VARIOUS MOUSE STRAINS IN THEIR RESPONSE TO LPF

Mouse strain	First preparation		Second preparation	
	Mean WBC count ^a x 10 ³ (range)	Standard deviation	Mean WBC count ^a (range)	Standard deviation
Swiss - RIV	10.7 (6.2-15.0)	28%	n.t.	-
Swiss - TNO	17.4 (8.5-29.0)	39%	45.0 (33.0-68.4)	22%
<u>NIH - RIV</u>	14.3 (12.2-15.8)	<u>8%</u>	66.0 (53.0-86.2) ^b	<u>13%</u>
Balb/c - TNO	10.4 (7.0-14.0)	25%	35.5 (24.0-61.2)	34%
C57 black - TNO	11.8 (8.6-17.0)	20%	n.t.	-
CDI - RIV	19.9 (15.6-23)	18%	n.t.	-
N - TNO	16.3 (10.8-21.2)	21%	50.0 (40.2-78.5)	22%
O ²⁰ - TNO	11.8 (7.7-15.8)	24%	45.0 (28.5-54.5)	16%

^a WBC = white blood cells; n.t. = not tested.

^b Group of five mice.

Annex I

On the basis of these experiments the NIH - RIV mouse strain was selected for the determination of LPF activity.

2. Isolation and purification of LPF

Supernatant fluids of B. pertussis (strain 134) cultures were used as starting material for the isolation of LPF. After harvesting of the bacteria by centrifugation the still slightly turbid culture supernatant was acidified to pH 3.5-4. After 48 hours the sediment had settled down. The supernatant was siphoned off and discarded. The remaining suspension was centrifuged. The sediment was washed once with PBS of pH 3.5 and extracted with a hypertonic buffer as prescribed by Sato. This concentrated LPF solution still contains a considerable amount of endotoxin as was demonstrated in the Limulus lysate test. Most of it can be removed by sucrose density ultracentrifugation. Further purification of the LPF will also be tried by ammoniumsulfate fractionation, Pevikon block electrophoresis or gelfiltration in hypertonic buffer.

3. Determination of the activity of a semi-purified LPF preparation, obtained from Sato

One ampoule containing 300 µg of lyophilized, partially purified LPF (fraction SDGC-1) was obtained from Sato. This material was isolated from B. pertussis cells cultivated on solid medium. The purification procedure consisted of ammoniumsulfate precipitation, starch block electrophoresis and sucrose density gradient centrifugation. The contents of the ampoule were dissolved in 1 ml of distilled water and volumes of 0.1 ml of four dilutions of this LPF solution were injected intravenously in groups of three NIH - RIV mice. The leukocyte counts three days after the injection of the indicated doses of protein are summarized in Table 2, together with the results obtained by Munoz, who tested the activity of the same lot of partially purified LPF (data obtained through Sato). In the same table the activity of an extract of the sediment obtained after acid precipitation of a culture supernatant with hypertonic buffer solution, prepared by ourselves (Bp 134-C), is given.

TABLE 2. DETERMINATION OF THE LPF ACTIVITY OF A JAPANESE, PARTIALLY PURIFIED LPF FRACTION (SDGC-1) AND OF A DUTCH CRUDE EXTRACT (Bp 134-C)

SDGC-1				Bp 134-C	
RIV ^b		Munoz ^a		RIV ^b	
Dose in µg of protein per mouse	Average WBC count	Dose in µg of protein per mouse	Average WBC count	Dose in µg of protein per mouse	Average WBC count
30	83 000	10	36 000	6	68 700
15	64 000	2	13 740	3	45 300
7.5	56 000	0.4	7 914	1.5	30 800
3.75	33 000	0.08	8 791		
0	9 000	0	8 955	0	9 000

^a Data obtained through the courtesy of Dr Sato.

^b Data obtained in the Rijks Instituut voor de Volksgezondheid.

In his papers Sato reports a specific LPF activity for this fraction of 23 000 units per mg of protein. As he defines one unit of LPF as the amount of protein giving rise to a leukocyte count of 32 000 cells per mm³, a dose of 0.044 µg of SDGC-1 should contain one unit. However, during lyophilization, he lost 50% of the activity.

Annex I

Nevertheless, in our hands, as in Munoz's, the preparation had a much lower specific activity. Even our crude extract had a higher specific activity.

It may be concluded that many more investigations will have to be done before a sufficient quantity of purified and stable LPF will be available for the production of a reference preparation.

4. Biological tests with HSF-free pertussis vaccine, prepared by Dr Tiru (Sweden)

From Dr Tiru two vaccines were obtained that were supposed not to contain HSF. These vaccines were prepared by repeated freezing and thawing of living pertussis cells. Further steps in the production included an ultracentrifugation at 85 000 x g and an alcohol precipitation.

These vaccines were tested in the mouse-protection, HSF, and LPF tests. According to the prescription of Dr Tiru, the vaccines PRB 200-2 and PRB 200-3 were diluted respectively ten- and twentyfold to contain about four protective units per ml. The results of the tests are summarized in Table 3.

TABLE 3. BIOLOGICAL ACTIVITIES OF TWO VACCINES
OBTAINED FROM DR TIRU

Vaccine	Mouse-protection test 25.4.74		HSF test 7.11.74			LPF test ^a 18.11.74	Limulus lysate test
	Potency (I.U./ml) (conf. lim. 95%)	Slope	Percentage death			WBC count	Positive after 30 minutes in a dilution
			1/1	1/5	1/25		
PRB 200-2 (diluted 1:10)	0.5 (0.05-2.1)	0.33	10%	5%	0%	18 600	>1/9 000
PRB 200-3 (diluted 1:20)	1.8 (0.3-8.2)	0.92	50%	0%	0%	14 400	>1/18 000
PRB 200-3 (diluted 1:2.5)	-	-	-	-	-	25 600	

^a LPF test: leukocytes were counted three days after the intravenous injection of 0.1 ml of the diluted vaccines.

Both vaccines show a relatively low potency. In addition, HSF and LPF are demonstrable. Relatively large amounts of endotoxin are still present.

It is stressed that the development of vaccines in which HSF, LPF, and endotoxin are separated from the protective antigen should remain the major goal in order to avoid side-effects in children. A better knowledge of the location of these factors in the bacterial cells may help to attain this objective.

REFERENCES

- Clausen, C. et al. (1968) Lymphocytosis and histamine sensitization of mice by fractions from Bordetella pertussis, J. Bact., 96, 1484-1487
- Cohen, H. et al. (1969) Relation between toxicity tests in mice and reactions in children using four lots of quadruple vaccine (DPT-polio). In: Symposia Series in Immunobiological Standardization, Vol. 10, Basel, Karger, pp. 53-62
- van Hemert, P. A. (1969) Specific properties of acid precipitated pertussis vaccine. In: Progress in Immunobiological Standardization, Vol. 3, Basel, Karger, pp. 297-301
- Kitagawa, M. et al. (1961) Chemical studies on cellular components of Hemophilus pertussis. II. Isolation of toxic lipopolysaccharide, J. Biochem., 49, 477-480
- Lehrer, S. B. et al. (1974) Extraction and partial purification of the histamine-sensitizing factor of Bordetella pertussis, J. Immun., 113, 18-26
- Levine, L. & Pieroni, R. E. (1966) A unitarian hypothesis of altered reactivity to stress mediated by Bordetella pertussis, Experientia, 15, 797-798
- Medical Research Council (1959) Vaccination against whooping-cough; final report, Brit. med. J., 1, 994-1000
- Morse, J. H. & Morse, S. I. (1970) Studies on the ultrastructure of Bordetella pertussis. I. Morphology, origin and biological activity of structures present in the extracellular fluid of liquid cultures of Bordetella pertussis, J. exp. Med., 131, 1342-1357
- Mota, I. et al. (1974) The mechanism of the adjuvant effect of Bordetella pertussis: the substance responsible for the selective enhancement of IgE antibody production, Int. Arch. Allergy, 47, 425-432
- Munoz, J. (1963) Symposium on relationship of structure of micro-organisms to their immunological properties. I. Immunological and other biological activities of Bordetella pertussis antigens, Bact. Rev., 27, 325-340
- Munoz, J. (1964) Effect of bacteria and bacterial products on antibody response, Advanc. Immunol., 4, 397-440
- Munoz, J. & Bergman, R. K. (1968) Histamine-sensitizing factors from microbial agents, with special reference to Bordetella pertussis, Bact. Rev., 32, 103-126
- Sato, Y. (1970) Isolation and some properties of the protective antigen from Bordetella pertussis. In: Symposia Series in Immunobiological Standardization, Vol. 13, Basel, Karger, pp. 214-220
- Sato, Y. & Arai, H. (1972) Leucocytosis-promoting factor of Bordetella pertussis. I. Purification and characterization, Infect. Immun., 6, 899-904
- Sato, Y. et al. (1973) Leucocytosis-promoting factor of B. pertussis. II. Biological properties, Infect. Immun., 7, 992-999
- Sato, Y. et al. (1974) Leucocytosis-promoting factor of B. pertussis. III. Its identity with protective antigen, Infect. Immun., 9, 801-810
- Thye Yin, E. et al. (1972) Picogram-sensitive assay for endotoxin: Gelation of Limulus polyphemus blood cell lysate induced by purified lipopolysaccharides and lipid A from Gram-negative bacteria, Biochim. biophys. Acta (Amst.), 261, 284-289
- Westphal, O. et al. (1952) Uber die Extraktion von Bakterien mit Phenol/Wasser, Z. Naturforsch., 7b, 148-155
- Zakharova, M. et al. (1970) Separation of biologically active components from Bordetella pertussis. In: Symposia Series in Immunobiological Standardization, Vol. 13, Basel, Karger, pp. 227-233

ANNEX II

THE DIFFERENT TYPES OF REACTION AFTER DPT-POLIO VACCINATION
AND THEIR FREQUENCY IN THE NETHERLANDS

by

Charlotte A. Hannik

In the Netherlands DPT-Polio vaccine is used nowadays for infants only. During the first year of life a complete course of four injections is offered free of charge by the Dutch Government: the three primary vaccinations at the age of three, four and five months, followed by the fourth or booster injection at 11-12 months. The vaccine is Al-phosphate-adsorbed and has to be injected intramuscularly.

Two to four hours after a DPT-Polio vaccination many babies show some malaise, a slight rise in temperature, and some tenderness at the site of injection. They recover rather suddenly after another two to four hours. This reaction is considered normal and is accepted as such by mothers and doctors.

In about one-third of the vaccinees this reaction is more serious with a high temperature lasting for a longer period and combined rather frequently with a local reaction, including redness, swelling, and substantial tenderness. These reactions are classified as minor reactions. They do not form a contraindication for further vaccination with the pertussis component, although some doctors in the Netherlands tend to drop this component easily. As part of these reactions are the result of an improper vaccination technique it is advisable to carry on with DPT-Polio vaccine, at least for the next injection.

Quite different clinically from these minor reactions are the so-called major reactions, the really serious complications. The two first cases were described by Madsen in 1933. At that time, however, blood-agar was used for vaccine production and, moreover, the vaccine was given therapeutically. Since then a substantial number of case reports and the results of several questionnaires have been published. Most attention was paid to cases with neurological symptoms. Berg gave his review of 107 cases in 1953 under the title: "Neurological complications of pertussis immunisation". Again, at that time, virological examinations were not performed routinely.

More recently, four cases with neurological symptoms after DPT-Polio vaccination were observed in children who at the same time were suffering from a primary infection with a virus which could itself have caused the neurological symptoms (Table 1). Two of the children with an encephalopathy resulting in permanent damage were suffering from a primary infection with Herpes simplex virus; one child with generalized convulsion and slight pleiocytosis in the spinal fluid had a coinciding infection with Coxsackie B5 virus; and the fourth child, a girl with transient paresis of the N. abducens, had an infection with Echo 25 virus. The ages ranged from six months to three years. None of the children had a reaction after the first or the second vaccination. The time interval between the vaccination and the first symptoms was rather long: for the child with the convulsion about 20 hours, for the others two and three days.

In the past such complications would have been imputed to the vaccine alone. Now, however, that blood-agar is no longer used for vaccine production, the vaccine is given prophylactically only, and virological examinations can be performed routinely, a careful analysis of each report is needed to obtain information about the different types of reaction. This was started in the Netherlands with the introduction of the DPT-Polio vaccine at the end of 1962. Every case reported to this Institute has been analysed and recorded.

Annex II

The following is a review of all the cases reported in the last 12 years.

Shock or collapse

Some hours after vaccination the children suddenly turn ashen, feel cold and clammy, and cease to react.

Table 2 shows that the cases occurred mainly in young children and after the first vaccination. With two exceptions, all reactions appeared within six hours after vaccination. One child showed a time interval of seven hours after the third injection, and another an interval of 12 hours after the fourth injection.

Because of this consistent pattern and particularly because of the short time interval, this reaction was considered to be vaccine-induced.

Convulsions or fits

The pattern in this group (Table 3) differs from that in the shock group in that there is more variation in age as well as in the order of injection. Moreover, the time interval ranges from 30 minutes to three days.

Although the children in this group have a convulsion or fit in common, it is clear that this group is not a homogeneous one (Fig. 1).

Undoubtedly some of the older children reacting with a high temperature and a convulsion to the booster vaccination had a febrile convulsion, i.e. a reaction to the vaccine-induced temperature rather than a direct reaction to some toxic activity in the vaccine itself.

On the other hand, some of the younger children with a family history positive for neurological disorders might have had a first convulsion spontaneously.

Fig. 2 presents these children according to the history of neurological disorders in the near family (father, mother, brothers, and sisters) or in the near family of the father or mother. The incidence of neurological disorders in the family is remarkably high.

Nearly half the children with a convulsion after vaccination had a positive history compared with 19% of the children in the shock group (Table 4). Especially striking is the occurrence of neurological disorders in the near family: 11 of 34 cases of convulsions with a known history - or about one-third of the cases - had a positive history compared with four of 47 cases of shock. If the shock group is considered as a control group these findings support the advice given by Cockburn as long ago as 1953 that children from families with a history of convulsions should not be vaccinated against pertussis.

On the other hand, three children with a father or mother with epilepsy showed no reaction after the four DPT-Polio vaccinations and one child with a febrile convulsion at the age of nine months showed no reaction after the fourth DPT-Polio vaccination.

Cerebral symptoms

The complication most to be feared after pertussis vaccination is encephalopathy. Mention has already been made of two cases of encephalopathy in children with a coinciding infection with Herpes virus. During the past 12 years another four cases have been reported in which the virological examination gave negative results or was not performed at all (Table 5).

Annex II

Again, the cases occurred in young children aged 2-6 months, three of whom reacted at the first vaccination. The time interval was rather long - up to three days after vaccination.

The first child started convulsions within 24 hours while her temperature was normal. The convulsions increased in frequency and duration but could be stopped in hospital with simple therapeutics. After one week she was blind and deaf. In about six weeks she recovered completely. The composition of the spinal fluid was normal.

The second child was listless on the second day after vaccination but had a normal temperature. The next morning he was found with hemiparesis. Two years later there was still a slight paresis.

The third child had screaming attacks on the second day after vaccination. One day later, while his temperature was normal, he started convulsions which could be stopped only with the most intensive therapy. He did not regain consciousness and died during the second period of apnoea on the fourth day after vaccination. There was a slight rise in the cell count of the spinal fluid.

The fourth child went to sleep quite contentedly after his first feeding on the second day after vaccination. Two hours later a peculiar colour of the skin was noticed and the mother took him to the family doctor. When he saw the child, he had him driven immediately to the hospital for emergency admittance. During the drive the child started tonic convulsions and half an hour after arrival he was dead.

Persistent screaming

This type of reaction is not reported spontaneously. However, once the physician is informed many cases are reported.

Table 6 shows a pattern similar to that in the shock group: young children reacting within six hours, and mainly after the first vaccination.

Experience has shown that too much reliance on the story of the mother can be misleading for diagnosis. A number of mothers never use the word "crying": their child only "roars" or "screams". This is especially true of young mothers confronted with the first real illness of their first child, caused by the vaccination. Anxiety leads them to exaggerate the symptoms. Moreover, any child with a tender local reaction can be made a persistent screamer by continuous handling, walking, and even shaking.

Furthermore, there are several types of persistent screaming, the extremes being crying with screaming attacks, which is screaming but not persistent, and crying loudly for hours on end, which is persistent but not screaming. As a result, no diagnosis is more difficult than that of persistent screaming.

Once it was known what reactions or complications occur after vaccination, the next aim was to establish their frequency. With that intention a survey was started in the province of Zuid-Holland. The doctors and nurses of all baby clinics were informed about the different types of reactions and were requested always to inquire about reactions after the previous vaccination before giving the next one. As soon as a reaction was suspected it had to be reported to this Institute so that a consultation could be arranged with the mother. As all vaccinations in the Netherlands are registered it is possible to obtain at least an idea of the frequency of the reactions.

The frequency of shock and convulsion is presented in Table 7.

Annex II

It should be pointed out that not all baby clinics in Zuid-Holland are reliable reporters. The most reliable data are those of the city of the Hague and the numbers are surprising: one in 3500 vaccinated children reacted with shock and one in 2150 with convulsion. This frequency is about the same as that indicated by Ström in 1960, although his findings were based on uncontrolled questionnaires and have quite rightly been criticized. Again, during the United Kingdom field trials in 1956 and 1959, Cockburn observed a frequency of 14 convulsions in 46 000 children, or one in 3300. Although not all convulsions can be considered to be vaccine-induced, the reported frequency of these reactions is certainly remarkably high.

During the 12 years of analysis a periodicity was observed in the frequency of the major reactions (Fig. 3).

The survey in Zuid-Holland started at the end of 1968 and the Hague started active reporting one year later. The number of miscellaneous cases, mainly serious minor reactions, has remained at much the same level. Therefore, the periodicity of shock and convulsion must be real and not due to periodic under-reporting.

This could be related to some activity in the vaccine that is not detected by routine control testing, as all vaccines fulfil the WHO and United States of America requirements for toxicity.

Summary and conclusions

The different types of reactions observed in the Netherlands after DPT-Polio vaccination have been described. The estimated frequency of shock is about one in 3500 vaccinated children. The frequency of all types of convulsions, starting within three days after vaccination, is about one in 2150 vaccinated children; this is of the same order as that observed by Cockburn in the United Kingdom field trials in 1956-1959 and by Ehrengut (1974) in Hamburg.

The children reacting with a convulsion after DPT-Polio vaccination showed a rather high incidence of neurological disorders in the near family. Therefore, neurological disorders of father, mother, brothers, or sisters are a contraindication for pertussis vaccination.

Annex II

TABLE 1. PRIMARY VIRUS INFECTION COINCIDING WITH DPT-POLIO VACCINATION

Age	Order of injection	Interval	Diagnosis	Virus	Spinal fluid
6 months	Third	2 days	Enceph.	Herpes	83/3 cells
3 years	Fifth	3 days	Enceph.	Herpes	19/3 cells
15 months	Third	24 hours	Febr. conv.	Coxs B 5	34/3 cells
11 months	Fourth	2 days	Paresis N VI	Echo 25	N.D.

TABLE 2. SHOCK AFTER DPT-POLIO VACCINATION

Age	Order of injection				Total number of cases
	First	Second	Third	Fourth	
2 months	4	-	-	-	4
3 months	23	-	-	-	23
4 months	4	6	1	-	11
5 months	-	2	5	-	7
6 months	-	1	-	-	1
11 months	-	-	-	2	2
16 months	-	-	-	1	1
Total	31	9	6	3	49

TABLE 3. CONVULSION AFTER DPT-POLIO VACCINATION

Age	Order of injection				Total number of cases
	First	Second	Third	Fourth	
2-6 months	14	7	5	-	26
7-14 months	2	-	-	11	13
Total	16	7	5	11	39

TABLE 4. FAMILY HISTORY POSITIVE FOR NEUROLOGICAL DISORDERS OF CHILDREN VACCINATED WITH DPT-POLIO VACCINE

Type of reaction	Total number	Positive history		
		Total	Near family	Family father/mother
Convulsion	34	16 = 47%	11 = 32%	5 = 15%
Shock	47	9 = 19%	4 = 8.5%	5 = 10.5%
No reaction	83	7 = 8.4%	4 = 4.8%	3 = 3.6%

TABLE 5. CEREBRAL SYMPTOMS AFTER DPT-POLIO VACCINATION

Age	Order of injection	Interval	Virol. cultures	Spinal fluid
3 months	First	±1 day	N.D.	Normal
6 months	Third	3 days	Neg.	Normal
4 months	First	3 days	Neg.	N.D.
2 months	First	2 days	Neg.	N.D.

TABLE 6. PERSISTENT SCREAMING AFTER DPT-POLIO VACCINATION

Age	Order of injection			Total number of cases
	First	Second	Third	
2 months	6	-	-	6
3 months	26	3	-	29
4 months	5	2	1	8
5 months	-	1	2	3
6 months	1	-	-	1
Total	38	6	3	47

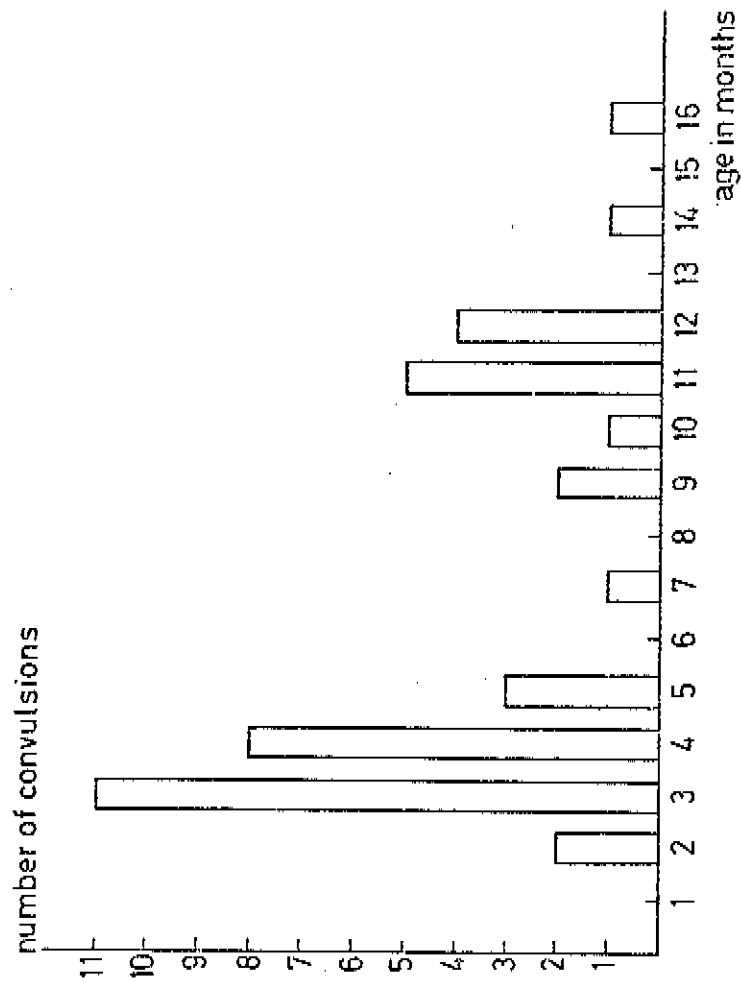
Annex II

TABLE 7. FREQUENCY OF SHOCK AND CONVULSION
AFTER DPT-POLIO VACCINATION

Period of observation	Estimated No. vaccinated children	No. of reactions	
		Shock	Convulsion
1970-1974	The Hague 28 000	8 = 1:3500	13 = 1:2150
1969-1974	Z.Holland 190 000	11 = 1:17 270	6 = 1:31 660

Figure 1

Convulsions after DPT - Polio vaccination

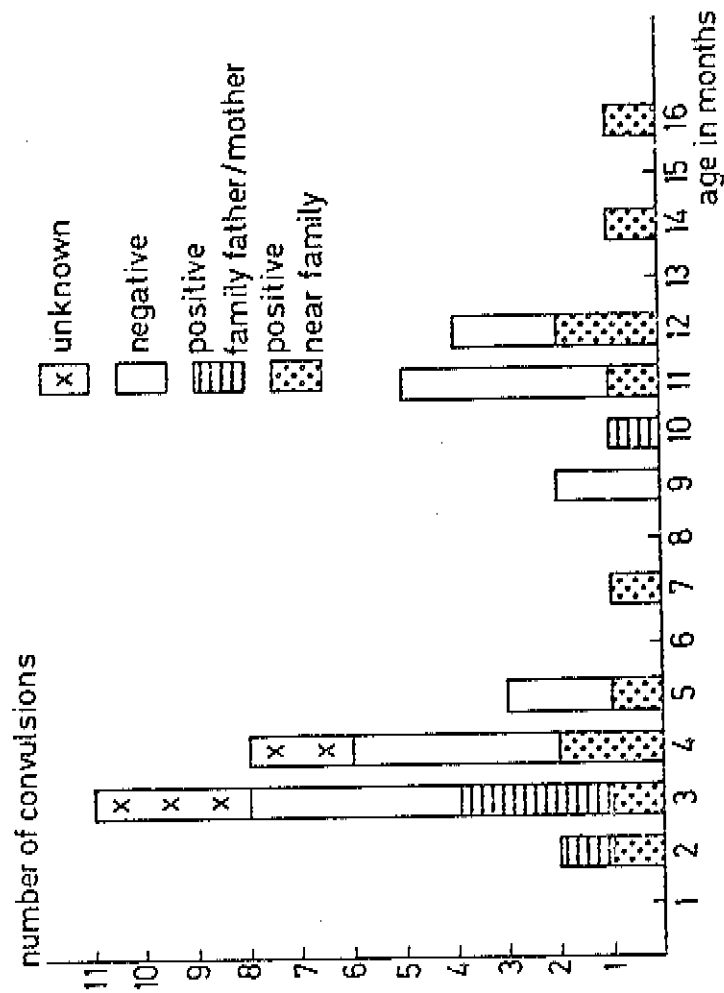


1533

R.V. B.P.

Figure 2

Family history of neurological disorders of children with a convulsion after DPT - Polio vaccination

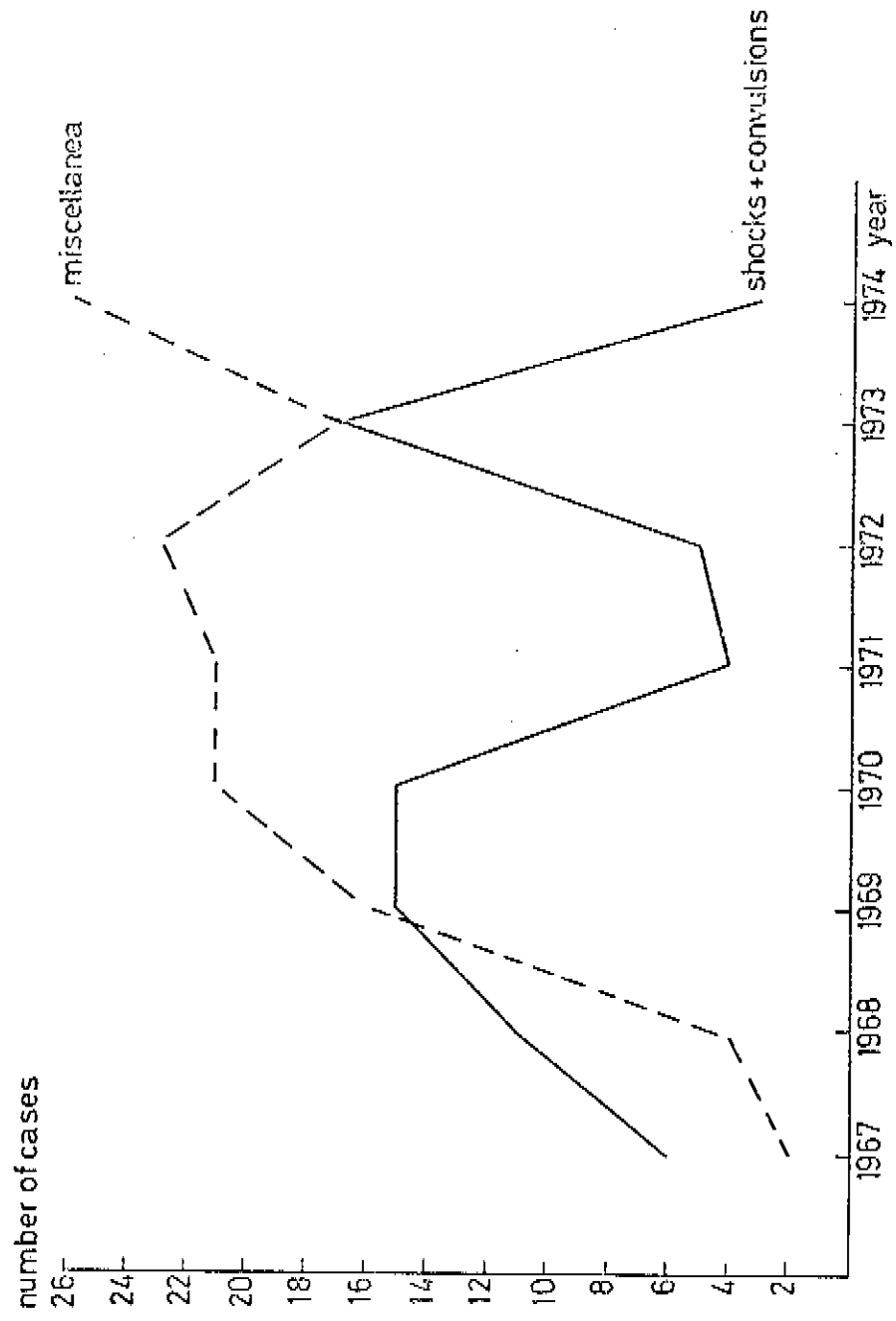


3538

R.V. B.P.

Figure 2

Periodicity in occurrence of reactions after DPT Polio - vaccination



R.I.V. B.P.

555A

ANNEX III

PHARMACOLOGICAL PHENOMENA OCCURRING
AFTER PERTUSSIS VACCINATION

by

Charlotte A. Hannik

It was in 1948 that Parfentjev & Goodline first reported the histamine-sensitizing activity of pertussis vaccine. Struck by the resemblance of the histamine shock in pertussis-vaccinated mice to the insulin shock, Parfentjev & Schleyer (1949) determined the bloodsugar level of mice, four days after vaccination and after a fasting period of five hours, and found a hypoglycaemia of about 55% of the normal value. In addition, the injection of a small dose of histamine, as used for histamine challenge in vaccinated mice, did not influence the blood-sugar level of control mice, but lowered the bloodsugar of vaccinated mice a further 15%.

Although HSF is an antigen against which an antiserum can be prepared (Maitland et al., 1955), the histamine-sensitizing activity is a pharmacological phenomenon. When the immune response of mice is suppressed by total body X-irradiation (Fishel, 1956) or by the administration of an immunosuppressive drug such as cyclophosphamide (Blake, 1969) the histamine sensitivity induced by pertussis vaccination is not inhibited.

Most strains of mice and rats are naturally resistant to histamine. For that reason Stronk & Pittman (1955) vaccinated rabbits, which, like men, are naturally very sensitive to histamine. However, the rabbits became even more resistant to histamine and did not show hypoglycaemia.

Protection of pertussis-vaccinated mice from the lethal effects of histamine is easily obtained by the administration of antihistamines a short time before the histamine challenge (Parfentjev & Goodline, 1948; Halpern & Roux, 1949; Kind, 1953).

Cortisone, an adrenal steroid effective in various allergic conditions, significantly inhibited the fatal effects of histamine (Kind, 1953).

Dibenzyline (DBZ), an α -adrenergic blocking agent, but also a potent antihistamine, gave complete protection against histamine death (Kind, 1954).

In none of these investigations were the bloodsugar levels determined. Whereas it is relatively easy to protect pertussis-vaccinated mice from histamine death, it is more difficult to influence the hypoglycaemia.

The most simple way to elevate bloodsugar is to administer glucose. However, exogenous glucose is effective in vaccinated mice only if it is administered during the first three hours after vaccination (Bergman & Munoz, 1969); thereafter, no further rise in bloodsugar is observed (Fishel & Szentivanyi, 1963).

Glucagon, the pancreatic hormone that produces hyperglycaemia in control mice, is not effective in vaccinated mice (Szentivanyi et al., 1963).

Another, rather drastic, method of inducing hyperglycaemia is to destroy all pancreatic β -cells, the cells that produce insulin. This can easily be achieved by injecting alloxan, which induces an irreversible alloxan diabetes. The administration of alloxan, some days before or after vaccination, produces a slightly higher bloodsugar level than in vaccinated controls, but the level is still considerably lower than in non-vaccinated, alloxan-treated mice and rats. This effect seems to be dose-related. The vaccinated animals treated with

Annex III

the high dose of alloxan showed some protection against histamine death and anaphylactoid shock (Parfentjev & Schleyer, 1949; Thompson, 1961; Ganley, 1962; Fishel & Szentivanyi, 1963; Gulbenkian et al., 1967).

A fourth method of inducing hyperglycaemia is to inject adrenalin (epinephrine). The rise in bloodsugar after the injection of adrenalin is significantly less in vaccinated mice and rats than in control animals (Fishel & Szentivanyi, 1963).

The results of the alloxan experiments are suggestive of a relationship between hypoglycaemia and histamine sensitivity. Stronk & Pittman (1955), however, noted that an undetoxified vaccine produced a greater degree of hypoglycaemia than the same but heated vaccine, while the histamine sensitization was not altered.

Bergman & Munoz (1969) demonstrated that hypoglycaemia starts earlier after vaccination than histamine sensitivity and lasts longer. Moreover, an insulin-induced hypoglycaemia of the same degree as that induced by vaccination, or even greater, does not sensitize control mice to histamine. Finally, the administration of d.mannoheptulose, a short-acting diabetogenic agent, induces a considerable hyperglycaemia in vaccinated mice but does not protect them against histamine death.

Histamine sensitivity and hypoglycaemia seem to be two separate coexistent phenomena occurring after pertussis vaccination.

Szentivanyi & Fishel started by investigating the histamine-sensitizing activity.

First they demonstrated that the metabolism of histamine was not altered by vaccination (Fishel et al., 1962). They concluded that "if the handling of histamine by the body as a whole" is not altered by vaccination, the induced histamine sensitivity must be the result of a "localized hyper-reactivity of some particular cells", in this case the target cells for histamine. Therefore, they directed their attention to the autonomic nervous system.

The functional entity of the autonomic nervous system is the neuron, consisting of a cell-body and an axon with a complex of neurites, the terminal, which may be compared with telephone wires (Fig. 1). The conduction through the axon is by potential differences, whereas the conduction from one neuron to another occurs chemically at a specific locus, the synapse. On their way from the central to the effector cells all autonomic nerves are interrupted by a synapse, situated in a ganglion. The chemical agent or neurotransmitter in the synapse between the pre- and post-ganglionic neurons is always acetylcholine. The neurotransmitter between the post-ganglionic nerve and the effector cells is either acetylcholine or noradrenalin. Thus the autonomic nervous system is divided into a cholinergic part and an adrenergic part, depending on the neurotransmitter in the final synapse.

The adrenergic transmitter - noradrenalin - is a catecholamine. Catecholamines are compounds containing a catecholnucleus and an amine group (Fig. 2). They are formed by adrenergic structures in the body from the amino acid tyrosine, which is normally present in the circulation. Dopa is the first catecholamine formed (Fig. 3). However, the use of the term "catecholamines" is usually reserved for dopamine, noradrenalin and adrenalin.

There are two main adrenergic structures; the chromaffin cells in the adrenal medulla, and the adrenergic neurons. In the chromaffin cells the amines serve as hormones. They are released into the blood stream and carry their message to every cell in the body. However, their signal is in code and only a few cells are able to decode the message. Wurtman (1973) compared hormones with radio waves: only appropriate tuners decode the waves into language. On the other hand, the amines released by the post-ganglionic adrenergic neurons reach special cells around the terminal only, i.e. in the analogy with telephone wires,

Annex III

only subscribers are reached (Wurtman, 1973). Together, radio and telephone - or hormones and neurotransmitters - form a regulatory system keeping the extracellular fluid in balance.

In 1948 Ahlquist concluded that the adrenergic receptor cells were divisible into two distinct types which he designated α and β :

noradrenalin acts mainly on the α -receptors

isoprenaline, an amine not produced in the body, acts mainly on the β -receptors

adrenalin acts on both α - and β -receptors.

The Szentivanyi-Fishel group (Fishel et al., 1962) first attempted to modify the histamine sensitivity with various autonomic drugs, starting with acetylcholine, adrenalin, isoprenaline, and noradrenalin. None of these agonists, injected 20 minutes before the histamine challenge, had any effect. Subsequently they tested adrenergic blocking agents, the antagonists. DBZ, an α -adrenergic blocking agent, gave complete protection. It has already been mentioned that this effect is most likely due to antihistamine activity. β -adrenergic blocking agents, however, such as DCI (dichloro-isoproterenol) and nethalide, actually increased the histamine sensitivity in vaccinated mice. Moreover, these agents made unvaccinated mice as sensitive to histamine as vaccinated mice. Thus pertussis-vaccinated mice behave in this respect as β -adrenergically blocked mice.

Another symptom of both pertussis vaccination and β -adrenergic blockade is the attenuated rise in bloodsugar after an injection of adrenalin (Szentivanyi et al., 1963; Fishel & Szentivanyi, 1963). This symptom is demonstrable as early as one hour after vaccination. Only a high dose of hydrocortisone was able to overcome this effect. It restored the adrenalin response in β -adrenergically blocked mice completely, whereas vaccinated mice showed a minimal, but significant, rise in bloodsugar.

Further similarities between vaccinated and β -adrenergically blocked mice are an increased sensitivity to immune haemolytic anaemia, hypothermia, bacterial endotoxin and peptone shock (Pieroni et al., 1971) and the failure of the free fatty acids and the lactic acid to increase after an injection of adrenalin (Keller & Fishel, 1967).

Following the Fishel group's unsuccessful attempt to protect vaccinated mice from histamine death by giving adrenalin 20 minutes before the challenge, Bergman & Munoz (1966) demonstrated that the timing of the adrenalin injection was of the utmost importance: if it was given intravenously 30-60 seconds after the challenge most mice survived; adrenalin injected 10 minutes before or five minutes after the challenge had no effect at all. When mice were highly sensitized against histamine the adrenalin injection was ineffective even with the right timing.

Because a larger dose of adrenalin is required to protect vaccinated mice against histamine death than is necessary to protect β -adrenergically blocked mice, it may be assumed that pertussis vaccine has some adrenergic blocking effect. In this context it is worth noting that the LD₅₀ of adrenalin increases as early as 24 hours after vaccination, reaching a maximum at seven days after vaccination (Denchev & Kosturkov, 1966).

Protection from histamine death of both vaccinated and β -adrenergically blocked mice can also be obtained with noradrenalin. Isoprenaline, however, protected the β -adrenergically blocked mice but not the vaccinated mice: the higher the dose of isoprenaline, the greater the mortality among vaccinated mice. Moreover, a mixture of noradrenalin (α -agonist) and isoprenaline (β -agonist) greatly reduced the protective effect of noradrenalin alone. Therefore, Bergman & Munoz (1971) concluded that although pertussis vaccination and β -adrenergic blockade had several symptoms in common, the mechanism of action was not identical.

Annex III

Further evidence for this conclusion is presented by Martorana et al. (1973). They found that the degree of histamine sensitization after β -adrenergic blockade was significantly lower than after pertussis vaccination. Besides, they found a divergence in symptoms after histamine challenge. It was observed that vaccinated mice showed marked depression and cyanosis before reaching the excitation stage and dying from respiratory arrest, 13-30 minutes after the histamine challenge; that blocked mice immediately showed excitation with jumping and convulsions and died within one minute after the injection; and that atropine, the antagonist of acetylcholine, was very effective in protecting β -adrenergically blocked mice from histamine death but did not affect the mortality of vaccinated mice.

In all these investigations adrenalin, which is mainly produced in the adrenal medulla, played an important role. This role is the more intriguing in view of the fact that extirpation of both adrenals (adrenalectomy) or even demedullation alone makes mice sensitive to histamine and various stresses. However, so far attempts to find adrenal abnormalities after vaccination have failed. The ability to maintain liver-glycogen at a moderately reduced atmospheric pressure is not altered by vaccination; under more severe anoxic conditions, however, the vaccinated mice die like adrenalectomized mice (Kind & Gansden, 1953). The weight of the adrenals and the baseline concentration of catecholamines are not influenced by vaccination (Szentivanyi et al., 1963), nor is the histology (Malkiel, 1956).

A new approach was made by Gulbenkian & Tabachnick (Gulbenkian et al., 1967, 1968; Tabachnick & Gulbenkian, 1969). They confirmed that vaccination induces hypoglycaemia and an attenuated hyperglycaemia response to adrenalin in mice (Szentivanyi et al., 1963) and demonstrated that rats react in the same way. After several investigations it was decided to continue with rats only because the experiments with mice showed a comparatively large variation. In their investigations neither the level of plasma free fatty acids (ffa) nor the adrenalin-induced elevation of this level was influenced by vaccination.

Struck by the divergence in the adrenalin response in vaccinated rats, namely, an attenuation of the hyperglycaemia and a normal elevation of the plasma ffa, they decided to study the ffa mobilization in vitro. For this test both epididymal fat pads were removed from anaesthetized rats and chopped into small pieces, and a fixed amount was incubated in a medium for two hours. By this method it was demonstrated that fat pads removed from vaccinated rats showed a marked ffa release of the same degree as that observed in normal fat pads after adrenalin had been added to the medium. This effect was decreased by adding the β -adrenergic blocking agent DCI; almost complete inhibition was reached by adding insulin to the medium.

Insulin is capable both of inducing hypoglycaemia and inhibiting lipolysis. Because the remarkable lipolysis in vitro after vaccination was assumed to be the consequence of a changed insulin level in vivo, plasma insulin levels were determined. The levels appeared to be considerably elevated. The observation that alloxan, given 2-3 days before vaccination or one day after vaccination, returned the insulin level to the same concentration as that observed in alloxan-treated control rats confirmed the pancreatic origin of the insulin. The biological activity was demonstrated by the hypoglycaemia observed in control rats after an injection of plasma derived from vaccinated rats.

In order to determine whether the increased insulin level was mediated via the pituitary gland or the adrenals, hypophysectomized and adrenalectomized rats were vaccinated. Only 10% of the animals survived the first 24 hours after vaccination. Of these surviving animals the hypophysectomized rats showed elevated insulin levels; the adrenalectomized rats did not. This finding suggests that the adrenals may have played a role in the rise of the insulin levels after vaccination.

Surprising was the finding that adrenalin even increased the already elevated plasma insulin level in vaccinated rats and the β -adrenergic agonist isoprenaline produced an even

Annex III

greater increase. Both these effects were markedly attenuated by the β -adrenergic blocking agent MJ-1999, which by itself did not alter the insulin level of vaccinated rats. Moreover, theophylline, enhancing the action of catecholamines by the inhibition of phosphodiesterase, also increased the already elevated insulin levels after vaccination more than tenfold. From this and further agonist and antagonist studies Tabachnick & Gulbenkian concluded that pertussis vaccination not only enhanced the β -adrenergic receptor activity but also the responsiveness of the pancreatic β -cells.

Conclusion

Following investigations of histamine sensitivity and hypoglycaemia after pertussis vaccination, as well as of β -adrenergic blockade, the latest concept is that pertussis vaccination induces enhanced β -adrenergic receptor activity and responsiveness of the pancreatic β -cells, expressed clinically in a marked elevation of the insulin level combined with a moderate hypoglycaemia.

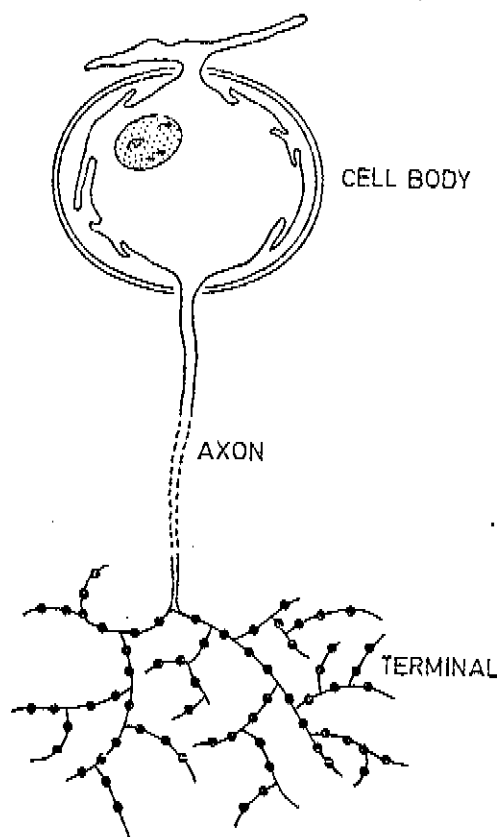
REFERENCES

- Ahlquist, R. P. (1948) A study of the adrenotropic receptors, Amer. J. Physiol., 153, 586-600
- Bergman, R. K. & Munoz, J. (1966) Protection against histamine shock by catecholamines in Bordetella pertussis-treated, adrenalectomized, or adrenergic blocked mice, Proc. Soc. exp. Biol. Med., 122, 428-433
- Bergman, R. K. & Munoz, J. (1969) Hypoglycaemia and its relationship to histamine sensitization in mice (33800), Proc. Soc. exp. Biol. Med., 131, 42-46
- Bergman, R. K. & Munoz, J. (1971) Effects of epinephrine, norepinephrine and isoproterenol against histamine challenge in Bordetella pertussis-treated and β -adrenergic blocked mice, Life Sci., 10, 561-568
- Blake, H. E. (1969) Inability of cyclophosphamide to inhibit the action of pertussis HSF in mice, Canad. J. Microbiol., 15, 1463-1464
- Denchev, V. & Kosturkov, G. (1966) Studies on the development and mechanism of increased tolerance to adrenaline in mice immunized with Bordetella pertussis vaccine, Z. Immun. Forsch., 131, 247-253
- Fishel, C. W. (1956) Host-parasite relationship in experimental pertussis. 1. Histamine-sensitizing and protective activities of pertussis vaccine in mice, J. infect. Dis., 99, 44-55
- Fishel, C. W. et al. (1962) Sensitization and desensitization of mice to histamine and serotonin by neurohumors, J. Immun., 89, 8-18
- Fishel, C. W. & Szentivanyi, A. (1963) The absence of adrenaline-induced hyperglycaemia in pertussis-sensitized mice and its relation to histamine and serotonin hypersensitivity, J. Allergy, 34, 439-454
- Ganley, O. H. (1962) Studies on the prevention of sensitization by Bordetella pertussis in alloxan diabetic mice, Canad. J. Biochem., 40, 1179-1183
- Gulbenkian, A. et al. (1967) The effect of altered carbohydrate metabolism in pertussis-sensitized mice on anaphylaxis, Biochem. Pharmacol., 16, 783-792
- Gulbenkian, A. et al. (1968) Metabolic effects of pertussis sensitization in mice and rats, Endocrinology, 83, 885-892
- Halpern, B. N. & Roux, J. (1949) Interférence entre l'immunisation par l'Haemophilus pertussis et l'intoxication histaminique, C.R. Soc. Biol. (Paris), 143, 923-927
- Keller, K. F. & Fishel, C. W. (1967) In vivo and in vitro manifestations of adrenergic blockade in Bordetella pertussis-vaccinated mice, J. Bact., 94, 804-811
- Kind, L. S. (1953) Inhibition of histamine death in pertussis-inoculated mice by cortisone and neoantergan, J. Allergy, 24, 52-59
- Kind, L. S. & Gadsden, R. H. (1953) Adrenal function tests in mice sensitized to histamine with Haemophilus pertussis vaccine, Proc. Soc. exp. Biol. Med., 84, 373-375
- Kind, L. S. (1954) Inhibition of histamine death in pertussis-inoculated mice by dibenzylamine, an adrenergic blocking agent, J. Allergy, 25, 33-35
- Maitland, H. B. et al. (1955) The histamine-sensitizing property of Haemophilus pertussis, J. Hyg. (Lond.), 53, 196-211
- Malkiel, S. (1956) Anaphylactic shock in the mouse vaccinated with Haemophilus pertussis, J. Allergy, 27, 445-449
- Martorana, P. A. et al. (1973) Differences between B. pertussis and propranolol induced histamine hypersensitivity in mice, Canad. J. Physiol. Pharmacol., 51, 102-107

Annex III

- Parfentjev, I. A. & Goodline, M. A. (1948) Histamine shock in mice sensitized with Hemophilus pertussis vaccine, J. Pharmacol. exp. Ther., 92, 411-413
- Parfentjev, I. A. & Schleyer, W. L. (1949) The influence of histamine on the bloodsugar level of normal and sensitized mice, Arch. Biochem., 20, 341-346
- Pieronì, R. E. et al. (1971) Bordetella pertussis as a beta-adrenergic blocking agent, Int. Arch. Allergy, 41, 637-647
- Stronk, M. G. & Pittman, M. (1955) The influence of pertussis vaccine on histamine sensitivity of rabbits and guinea pigs and on the bloodsugar in rabbits and mice, J. infect. Dis., 96, 152-161
- Szentivanyi, A. et al. (1963) Adrenaline mediation of histamine and serotonin hyperglycemia in normal mice and the absence of adrenaline-induced hyperglycemia in pertussis-sensitized mice, J. infect. Dis., 113, 86-98
- Tabachnick, I. I. A. & Gulbenkian, A. (1969) Adrenergic changes due to pertussis: insulin, glucose and free fatty acids, Europ. J. Pharmacol., 7, 186-195
- Thompson, G. E. (1961) Alloxan and hypersensitivity, Nature (Lond.), 190, 822
- Wurtman, R. J. (1973) Biogenic amines and endocrine function, Fed. Proc., 32, 1769-1771

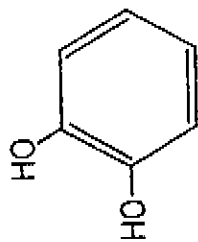
FIG. 1



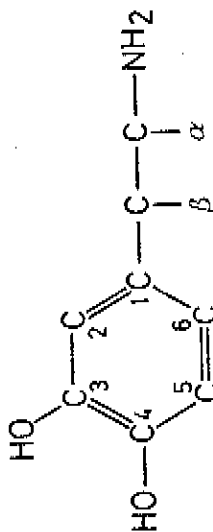
The Sympathetic Neuron (Adapted from Geffen LB, Livott
BG: Synaptic Vesicles in Sympathetic Neurons.
Physiol Rev 51:98-157, 1971)

Annex III

CATECHOLAMINES



catechol nucleus



catecholamine

from Richard J. Wurtman
The New England J. of Med., 1965, 273/12, 638

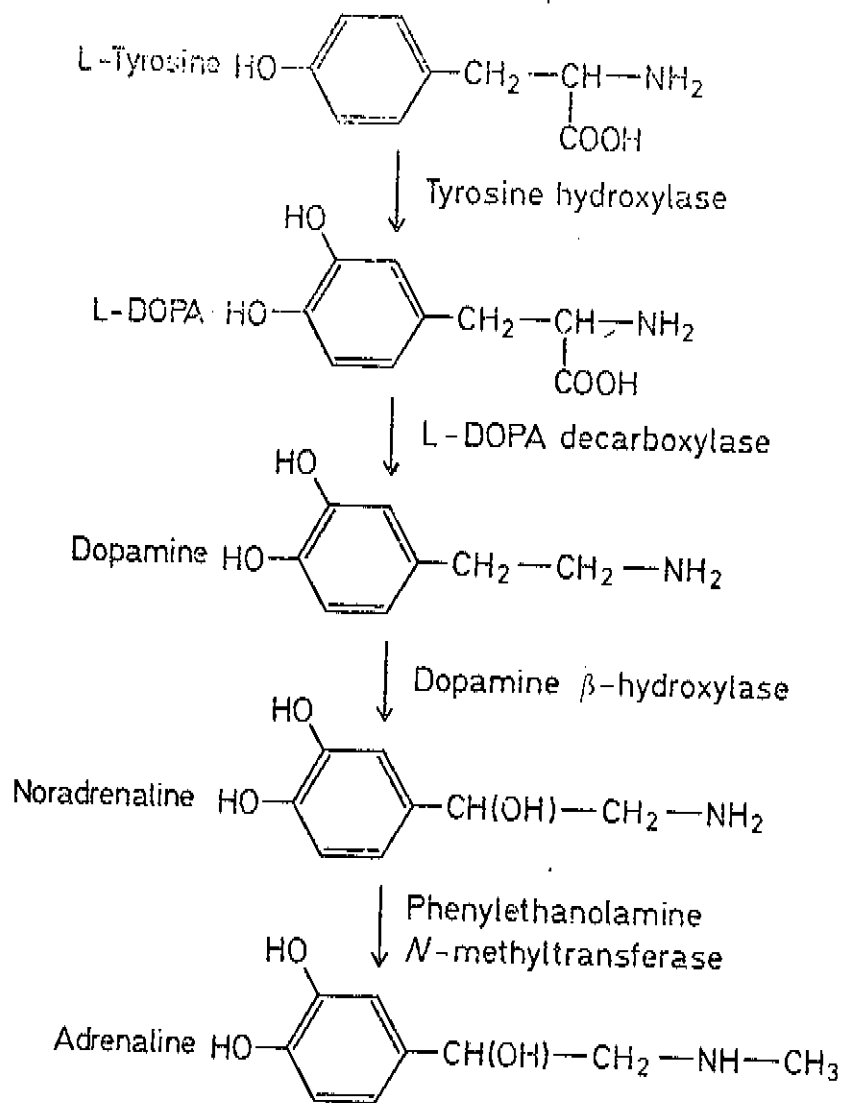
FIG. 2

SS14

SIV B.P.

FIG. 3

Intermediate stages in the formation
of adrenaline



from H. Blaschko
(Brit. Med. Bull.), 73, 29/2, 105