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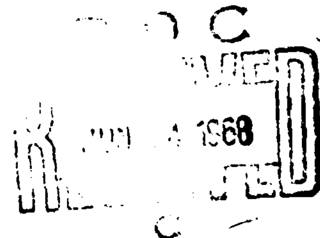

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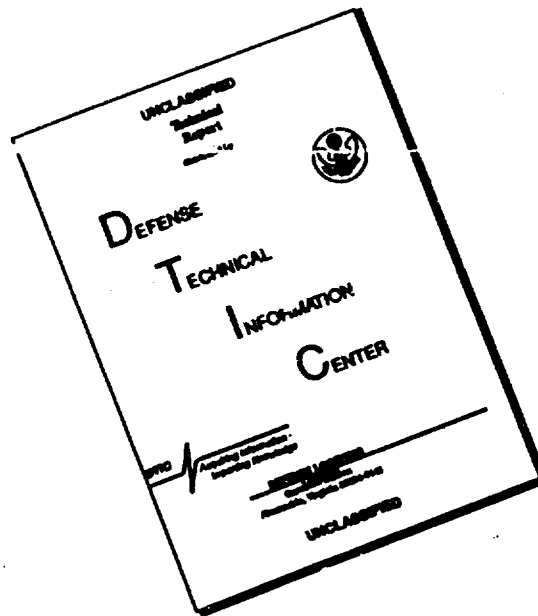

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#1755

PROBLEMS OF LIVE VACCINES IN COMBATING VIRUS DISEASES OF HUMANS

Translation No. T-415-1

MAY 1966

U. S. ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND

PROBLEMS OF LIVE VACCINES IN COMBATING VIRUS DISEASES OF HUMANS

(Following is the translation of an article by R. Siegert, published in the German language periodical, Zent. Bakt: 191, 1963, pages 21-36. Translation performed by Constance L. Lust.)

The peculiar problems that are connected with the use of live-vaccines in combating virus illness first really became evident during the development of the oral poliomyelites vaccine. From this task new principles were used and these in turn had to be used to explain the development of older vaccines. The practical experiences with live vaccines often ran far ahead of the known scientific knowledge. Therefore many basic answers must still be forthcoming on basic-principles. My report is limited to such problems which appeared to merit special emphasis in the area of pock, polio and measle vaccines. ~~This limited selection is justified because of numerous other reports in the present literature.~~

In artificial immunizations with live vaccines it is known that conditions of a subclinical infection exist. We use strains of pathogens that do not manifest the characteristics of illness. They must, however, be similar to actual pathogens in their replication and antigenicity in order to offer optimal immunity. This goal may be obtained in several different ways. It is possible that one can use antigenically related viruses of different specificity, for example, vaccinia virus with pock protection as is shown in the first figure, its derivation is disputed.

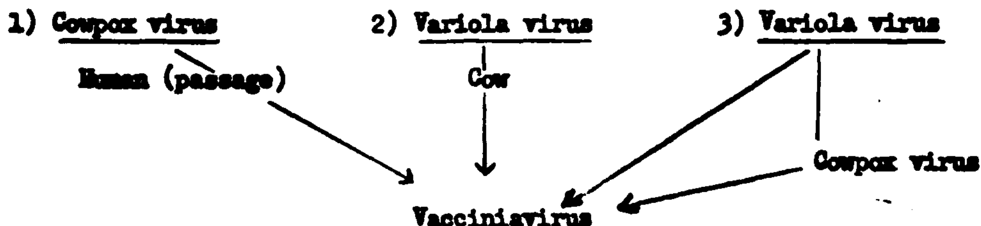

Figure 1. Derivation of Vaccinia Virus

Jenner originally protected man against variolavirus with cowpox virus. The transition to vaccina virus is to have taken place in the human lymph in subsequent human passage. Other authors assume that vaccinia is a modified variolavirus, which has lost its susceptibility for man because of animal passage (1,2,3). It is interesting that in the last 25 years it has no longer been possible to propagate variolavirus in the skin of animals. Also no more modifications appeared by monkeys, mice and egg passage (4-7). The change from variola to vaccine may be no more than the result of a selection, as was proved by Herrlich (8) who studied a freshly immunised variola patient during the Heidelberg

epidemic. Finally, it may have been that a special strain of variola changed int vaccinia through cowpox in the course of many generations (7). Today we have three independent types of viruses with similar properties (7,9,10,11).

In measle prophylaxis one also tried to use antigenic similarities for immunization purposes; as they exist between measles and Staupevirus (trans?) (12, 12a, 13). The protection of the apathogenic (for man) distemper virus was, however, unsatisfactory in trials in Panama (14). Enders-Ruckle proved more recently that miniavirus (?) grown in monkey kidneys is useful. This is identical to the human measle virus (15). It lost its virulence for monkeys after very few passages in human kidney cells; its antigenicity was not lost (16).

1) Edmonston-Strains (Enders/Boston) isolated from human kidney cell culture
Vaccines
A - 6x egg embryo + 14x chick fibroblasts
B - 12x egg embryo + 17x chick fibroblasts
Roxan - Vaccine A + 3-15x dog kidneys
Pitman-Moore - Vaccine A + 77x chick fibroblasts

2) USSR-58-Strain (Zhdanov/Moscow) from primary human amnion tissue culture
test trial - egg for hatching + chick fibroblasts + monkey kidneys
field trial - propiolacton treated monkey kidney

3) Leningrad-4-Strains (Smorodintsev/Leningrad) isolated by human tissue culture
test trial - 9-15x egg embryo + chick fibroblasts
field trial - egg for hatching + chick embryo + guinea pig kidneys

4) Toyoshima Strain (Okuna/Osaka) isolated from primary human amnion cell culture
vaccine - 28x egg for hatching (25x amnion + 3x allantoic cavity)

Figure 2. Derivation of strains of measle vaccines

It is known that good results are possible by passing the pathogens themselves through heterologous hosts whereby their virulence is diminished for humans. This is how yellow-fever vaccines are made (17, 18). Some polio strains were also attenuated by passing them through rodent brains, primate tissues or chick embryos. It appeared for a time that this process might have been suitable to produce the oral vaccine itself (19-22). These tedious procedures were of little meaning practically, since Sabin (23) used a better approach to successfully make oral vaccine. He isolated virus clones which were practically avirulent variants. These finally were used for the oral polio vaccine.

The derivation of the present-day measle vaccines are reviewed in figure 2.

Enders (24) first succeeded in adapting the Edmonston strain in "eggs for hatching" and in chick fibroblasts. This was associated with an attenuation of the virulence for monkey and man. However vaccine A still produced fever and exanthema in most sensitive children. Further passage did not lower the virulence, even if heterologous passage through dog kidneys was included. Meanwhile Schwarz (25) succeeded in attenuating the strain so far that only an inapparent infection was elicited. Russian and Japanese workers attenuated other strains and used these in field trials with results similar to those of Enders (26, 27, 28).

The most important prerequisite for the use of live vaccines is that the properties of avirulence and antigenicity remain stable. In order to detect qualitative alterations we required others, to correlate the genetic markers. They represent the functional equivalent of the nucleoprotein structures which are responsible for the maintainance of their identity. In figure 3 the "marker picture" of the vaccine strain and of wild strains of poliovirus are shown.

	Neurovirulence	Wild virus strain	Vaccine strain
	Chimps (intraspinal)	++	-
	Monkeys (intrathalamic)	++	-
	" (intraspinal)	++	+
in	" (intramuscle)	++	-
vivo	Viremia		
	Monkeys (intramuscular)	++	I - II + III -
	Human (oral)	+	I + II + III -
	d (Bicarbonate)	+	-
	rect/40 (temperature)	+	-
in	A ($AlCl_3$)	+	-
vitro	MS (stable cell line of monkeys)	+	-
	E (Elution)	+	-
	Cystine	independent	required
	seromarker	stable	stable

Figure 3. Markers for virulent polioviruses and avirulent vaccine strains.

It is most important to test the neurovirulence in monkeys. This shows the limits of virulence which are tolerated by man. Vivemia will be discussed later. In vitro markers are the different sensitivities of the strains to varying propagation conditions; low HCO_3-concentration = d marker (29), or elevated incubation temperature = T marker (30), or $AlCl_3$ (31). The different susceptibility of individual strains is used = MS marker (32, 33); or the resistance to physical-chemical influences = cystine marker (34). Surface properties of virus/particles may also be included (35) = Elution marker. Haas et al (36) worked extensively on the latter phenomenon. The serummarker should be the most stable marker. The neutralisation test by specific antiserum of McBride (37) is less dependable than the Wecker test (38), which measures the effect of the antibody on plaque-size and number.

The Edmonston strain of measle vaccine and the wild type virus are different in many markers; different cytopathic effects in different cell culture systems, hemagglutin formation, enzyme activity, as well as their temperature and UV resistance (39, 40-42).

If we want to study the stability of the vaccine viruses then we must concern ourselves with the genetic variants. The elucidation of its genetic basis is difficult, because we are not dealing with individual virus particles, but at best with the derivative of a part thereof. If the behavior of the variants is to be analysed in terms of the modern theory of evolution, we have to know the distribution of the genotype in the original and final population. The type and frequency of mutations as well as the effect of environment must all be considered.

In order to obtain this information, we require pure virus clones, which are clearly differentiated in their phenotypic markers. Clone-isolation may be done by plaque technique or by means of continuous dilution passages.

Supposedly the vaccine strains can replicate under lab conditions as well as in a vaccinated host. The progeny are genetically different from the original virus particles. With polio these differences are evident from the in vitro markers all the way to the return of full virulence in monkeys. This is seen most with strain III. The existance of neurovirulent variants is only noticed after injection directly into the brain of monkeys, because these components act selectively in this tissue. There are areas of sensitivity in the neurotropic spectrum, which are not dangerous if the infection starts in the intestine. The reversion to wild type occurrs either in a single mutation step or stepwise through partial reversion, as was observed with influenza (43-45) and poliovirus (46). Host induced changes (47, 48) may be seen phenotypically, even if genotypic changes were not excluded (49).

The antigen structure which was preferred as an index in the characterization of the vaccine and wild strains was thought to be particularly stable. New data seem to indicate that the antigen of Sabin strain type I in intestinal passage is less stable than that of type III or of the Lederle-strains (50, 51). Therefore, the serum marker is also not a dependable fingerprint for the origin of a strain.

The rate of change is limited by the number of loci in the genome, which control the properties. If it is controlled from only one gene locus, then it should be less stable as when the combined activity of several loci is involved (50). The different stability of some marker after intestinal passage is dependent on the mutation rate of the polio strains. The mutation rates are rather different for the different poliovirus strains (52). This fact should also be considered in selecting a strain for immunization purposes.

Mutations with new biological properties are known. This may also be achieved artificially by means of mutagenic agents. Schramm and co-workers (1958) have obtained good results with the nitrite experiment (54, 55, 56). The mode of its action was first explained by using tobacco mosaic virus as a model-system (57, 58).

Mutations with virulent and attenuated polio strains were also obtained by using nitric acid (59, 60). This treatment lead to a deamination of purines (adenine, guanine) and pyrimidine bases (cytosine) of RNA (61). In this process reversions may occur in genetic markers (59). In these experiments direct utilisation of naked RNA, as carriers of the genetic information was shown to be more successful than the effect on the intact virus particle (62).

Another way to make virus mutations may be at hand in the method described by Schramm (63). This method makes apurin-DNA. The purine bases, adenine and guanine may be split off easily with weakly acidic solutions, without breaking the chain of the molecules. According to Schramm (64) it may be possible to fill these gaps selectively with purines or pyrimidines and in this way make new nucleic acids which have unknown biological properties and functions.

We constantly use the concept of virulence without being able to define it. Virulence always represents a phenomenon of different origin and behavior for a cell of an organism. The mechanism of virulence of viruses in the final analysis involves the surface and metabolism of a host cell. It actively uses the host-cell's metabolism. To explain these events, the discovery of interferons, made by Isaacs and Lindemann, may prove to have an important role. Interferon is a cellular protein which acts on viruses and is responsible for interfering in virus growth and for providing resistance to cells after virus infections. Interferon synthesis is induced by the avirulent strains of measles and polio (66,67), which are actually very sensitive to its antiviral action. The fully virulent wild strains either actively circumvent this mechanism, or inhibit its activation in a cell (68). Perhaps this path will lead to a dependable virulence marker.

Now let us turn to the behavior of the vaccine viruses within an organism. Here we do not even know very much about the well-known vaccinia virus. Therefore we cannot answer the question of how the vaccine complications arise in the central nervous system. After a local reaction at the site of injection and a hasty viremia the vaccine-virus disappears. Maximal viremia occurs between day 5 and 7 (Herzberg, 69), and should not exceed 100 elementary particles per ml of blood. If virus is found after 10 days this is considered abnormal.(67, 70). It is now known whether viremia can occur 97-100 days after injection. Alivisatos (71) has made this claim, however, some doubt exists against this hypothesis as well as against his hypothesis of immature virus in the blood. We still do not know if and where replication takes place in the blood (72-74). Vaccinia

virus (Gino, 75) is separated out by the tonsils after 3-4 days. However, these findings could not be repeated recently (76), using modern culture techniques.

Vaccinia virus thus far has not been found in fluid or in brain during the normal course of vaccination (70, 74, 77-79). Whether the occassional evidence for virus during encephalitis vaccines is important pathogenically must still await further work (79, 80).

The measle vaccine strains are extensively attenuated in their ability to attach and invade a host, so that the infection occurs only unregularly in the normal manner (81, 82). Therefore the parenteral route of administration is preferred (83, 84). The Edmonton strain was only found twice in blood and once in the throat (81); the Leningrad strain was found more frequently (82). Either the latter is less attenuated or the cell system used to show its presence is more sensitive. Since virus is not released contact-infections have not been observed after measle vaccinations (85).

The measle vaccines despite their excellent immunization effect have not been used for other cases (83, 84, 86, 87), since about 80% of the vaccinated subjects show weak measle symptoms. It does not adversely influence chronic cases (83, 84, 88-90). Only leukemia acts differently (91, 92). Complicating factors after vaccination are usually secondary infections of the respiratory system (93, 94). Even though during real measles EEG-alterations are observed (95) this does not appear in vaccinated subjects even if they showed a fever spike (85, 95). Administering up to 10% gamma globulin suppresses the undesirable effects of the vaccine. It must be dosed properly and administered separately (97, 98). This does not alter immunization (99, 100).

A rather new problem has appeared; that viruses can influence chromosome morphology in mammals. In tissue culture herpes infection induces chromosomal aberrations and chromosomal breaks during metaphase of the initial cell division (101). Even though the measle virus can replicate in leucocytes (96) no deductions about lymphocytic leukemia can be made there from.

The purely hypothetical thought that potential carcinogen could be carried over with live vaccines is equally applicable for occasional impurities (103-106) of foreign viruses (eg. SV_{40}, Adeng 12 and 18) and for heteroploid cell-cultures which could possible cause a malignant transformation (107).

A serious problem is presented by the numerous viruses present in monkey kidney cells (108-110). To prove the presence of these viruses is difficult because the technology of these analyses is not far advanced (111) as was demonstrated by the Cutter-accident with Salk vaccine, or SV_{40}-virus.

The danger of impurities may be diminished by using substitute for monkey kidney cells, eg. primary human cells. This is usually not done

because of the danger of unknown human pathogenic viruses, like hepatitis virus. However, permanent cell lines, like Hela or Hep-2 cells, are usually free of viruses. These are not used extensively because of their altered chromosome picture, which resembles that of tumor cells. Hayflick and Moorehead (117) recommended using human embryonic kidney or lung cells for human vaccine production. These cells retain their euploid chromosome picture for a certain, limited, number of passages. One may completely omit mammalian cells and adapt pathogenic strains in chich embryos. This was done by Cox with polio type II and III. These are not completely free of "replicating" antigens (113, 114). I refer to a virus of the parainfluenza group which was found in embryos of numerous German chickin raisers by Enders-Ruckle (115).

It is possible to suppress or illuminate undesirable viruses in cell cultures by addition of immune sera, also differential inactivation with toluidine blue and visible light, or purifying with ion-exchangers. These procedures may affect the genetic stability of the vaccine and this must be taken into consideration.

Live vaccines should be free of impurities of inducing antigens, regardless of whether their pathogenicity is known or not. However, the specifications are variable today. For example, bacterial sterility is not guaranteed with pox vaccines. We recently investigated 12 vaccines. One French vaccine contained 300 germs/ml which belonged to 8 different types of bacteria and fungi. German vaccine preparations were also contaminated with antibiotic resistant Enterococci and strains of moulds. The pox vaccine was particularly in need of purification and control. This refers to its genetic basis, method of production, government testing and dose to be used for immunization prophylaxis.

I would like to now go into some of the problems of the oral poliovirus vaccine; intestinal resistance, viremia, and finally vaccination complications.

The oral vaccine induces, like the natural infection, a serological measureable immunity, which goes much higher than the Salk-vaccine. The oral vaccine leaves a local resistance to reinfection with the homologous type in the intestinal tract and mucous membranes. This is responsible for the suppression of the wild-type strain along with the interferance-mechanism. This intestinal resistance is thought to be a local cell immunity and should be independent of the humoral immunity (23, 116-118, 122).

In a vaccine trial with 95 children and adults we found (to be published soon) (119, 120) that a clear-cut separation between humoral and local immunity is not justified. This became apparent by comparing virus separation from vaccinated subjects with different prevaccination antibodies.

As may be seen in figure 4 practically all children and adults released the polip type I strain into throat and stool after feeding of about 300,000 "tissue culture" units of type I vaccine. If high pre-

vaccination titer of antibody was present the percentage liberated decreased. The differences shown are statistically significant. Above a middle titer no further effect was evident.

The close relationship of the humoral and intestinal immunity is made still clearer in figure 5. Here the "time to release" in the throat and stool in various groups is compared. The majority of fully receptive children released the vaccine virus at least by 18-28 days. With increasing prevaccination antibody level the time as well as the concentration was correspondingly lowered. Even with maximum titer a short term release was possible. The vaccine virus disappeared by 3 month after vaccination in our population at the latest. These, and other, results point to a close correlation between humoral and local immunity. Based on newer work, there remains little doubt that antibodies also act on the mucous membrane of the intestine (121-123).

Discussions still are heard about whether an age-dependent resistance influences the replication of the vaccine virus (124). If one looks at anti-body-negative, small children and adults then the release is actually reduced by about half in adults compared to the children.

As can be seen in figure 6 also, the age-dependent resistance is, however, an immunological phenomenon. In most adults antibody formation starts earlier and leads to higher titers than in children. Most adults had to release their polioantigen during the time that children were still inexperienced.

Like for the pathogenesis of polio the important viremia was not found for a long time, so it remained hidden for the oral vaccine. All attenuated strains appeared to have lost the ability for viremia as well as their neurovirulence. However, it was later found that the Lederle strain of type I and III (125, 126), the Fox strain (127) and the type II Sabin strain can all cause viremia (128-130).

A short-term viremia was observed for oral vaccine of type I (119, 120, 131), which was hard to determine because the methods lacked sensitivity. Viremia may be a prerequisite for stimulation of antibodies, as well as for reactions observed after vaccination.

The foreign reports, which claimed that no reactions or other complications were to be expected with the oral vaccine, were received with skepticism by us, since we assumed that no risk-free vaccine process existed. With the Sabin strains less risks were to be expected from the start, because of their very minimal newrovirulence.

Figure 7 helps explain the vaccination reactions and complications. A well-known scheme is presented; the relationships between the pathogenic processes and the clinical reactions of natural poliomyelitis. The phase of viremia and virus release into throat and stool is represented by the dotted area. Since the dose of virus in this experimental infection

was higher than under natural conditions, the virus release into throat and stool reached maximum after 2-4 days. Viremia also occurred correspondingly earlier. Also the incubation period of the vaccine reaction must be shorter. This was already observed by the many minor illnesses about which Lennartz (132, 133) and Wohlrab (134) have reported. Even if vaccine-damage occurred a shortening of the incubation period should be expected.

How should vaccination-damage be defined? If nothing is known the worst that would be expected are the usual polio symptoms; not only paralysis, but also a paralytic illness. The reduced neurovirulence must also be taken into account along with the shorter incubation period; on might expect early partial paralysis. Whether other trouble should be expected cannot be answered thus far.

A casual relationship between central nervous system disturbance and vaccination can only be proved if the vaccine virus can be isolated from the CNS. This has not been possible in any cases with the Sabin strains. We have to be satisfied with the uncertain decision guessing whether the polio vaccine virus can be released. Pette (135) recently offered some contrary views to those of Schaltenbrand (130) who claimed he had such cases. The most important objection is one that says that biologic basis for any conclusion is lacking. An important observation has been made by Kruecke (137). He isolated material from four vaccinated patients who died. The material gave symptoms of herd encephalities with characteristic markings in the area of the peripheral and CNS.

Difficulties in determing the meaning of experiments are not only encountered by clinicians and pathologists, but also virologists. The data that virus appeared in the throat and stool after vaccination is not evidence that a causal relationship exists between trouble and vaccination. It merely shows that the virus was released and that a complication may arise. It is more difficult for the virus tobe identified, either with the vaccine or wild strain, the later the isolation of the virus is undertaken after vaccination. Gelfand (138) recently pointed out that the interference mechanism may also play an important role in this process.

Despite all these reports, we believe that in the U.S.A. as well as in W. Germany in the spring of 1962 vaccine-damage was observed with a type I strain. To be sure, there were very few cases. A summary of material from several provinces is listed (see also article by experts of the German Society for Protection Against Polio; Deutsch Med. Wochschr. 88, 1821, 1963).

The suspicion was first aroused on clinical-epedemiological grounds. In Hessie, Baden-Wuentenberg no clinical cases of polio type I were observed in the months preceding the vaccination. Also no strains of wild type were isolated. As can be seen in figure 8, 9 cases of paralytic polio occurred in the spring of 1962 after the oral vaccine was administered

to 6 million participants. The cases were not in the same place. In all cases the vaccine-virus was isolated from stool and antibody elevations were determined. This meant that release had occurred. The incubation times were 5-13 days. One case which had previously received 3 Salk vaccinations started to show complications. From this child vaccine virus was isolated from stool, but simultaneously also Coxsachie virus B_4 was isolated from fluid. Furthermore a hypo-gamma globénámia existed which explains why antibody was not demonstrable. Since this involved a long paralysis, the basis for the damage was suspected to be derived from the vaccine.

The relatively numerous cases reported of facial paralysis are widely distributed. Among these were several cases of aseptic meningitis, which may also have been derived from vaccinations.

With these references I would like to end my report. The problems described illustrate the limited extent of our knowledge. We must admit that more questions remain unanswered than solved. The success of the live vaccines is not only the fruit of human research, but apparently some thanks must be given to nature for some favorable conditions.

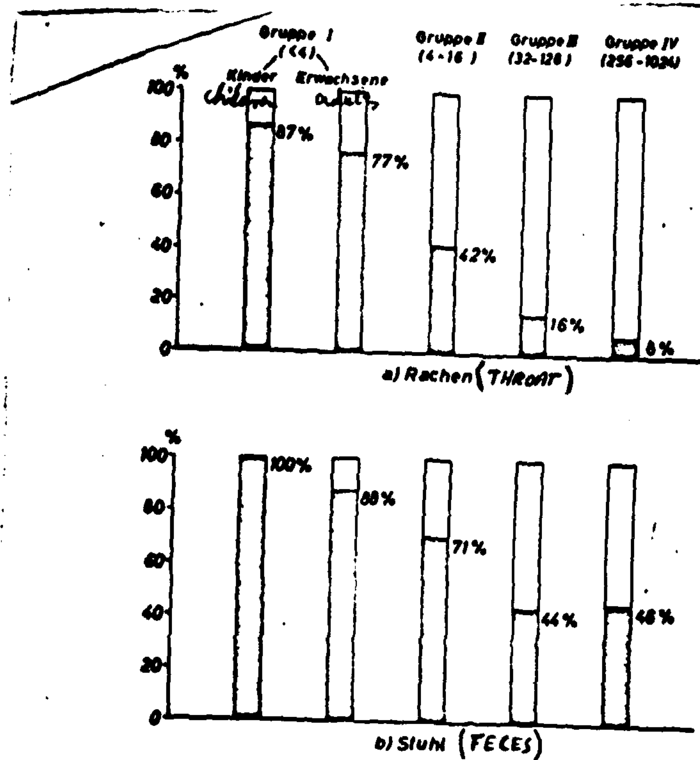

Abb. 4. Prozentsatz der Ausscheider (Poliotyp I – SABIN) in Rachen und Stuhl in Abhängigkeit vom praevaccinalen Antikörperspiegel.

Figure 4. Percentage of liberated virus (Polio type I-Sabin) in the throat and stool in dependence on the prevaccine level of antibody.

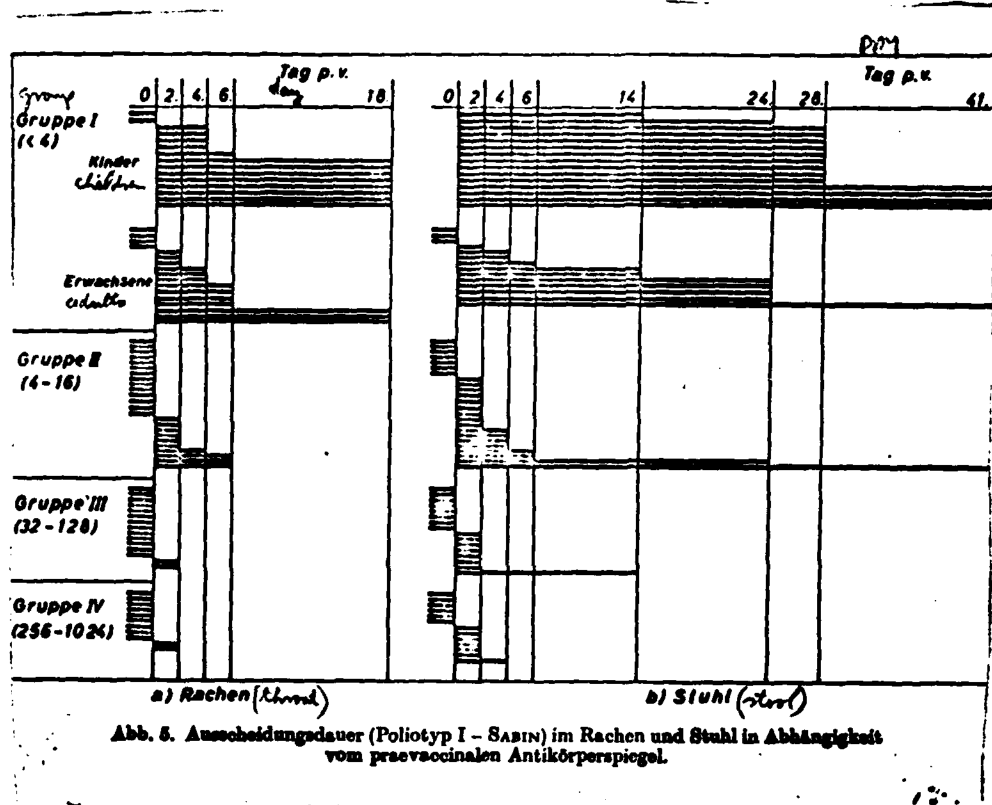

Abb. 5. Ausscheidungsdauer (Poliotyp I – SABIN) im Rachen und Stuhl in Abhängigkeit vom praevaccinalen Antikörperspiegel.

Figure 5. Time to release (poliotype I-Sabin) in throat and stool dependent on prevaccine antibody level.

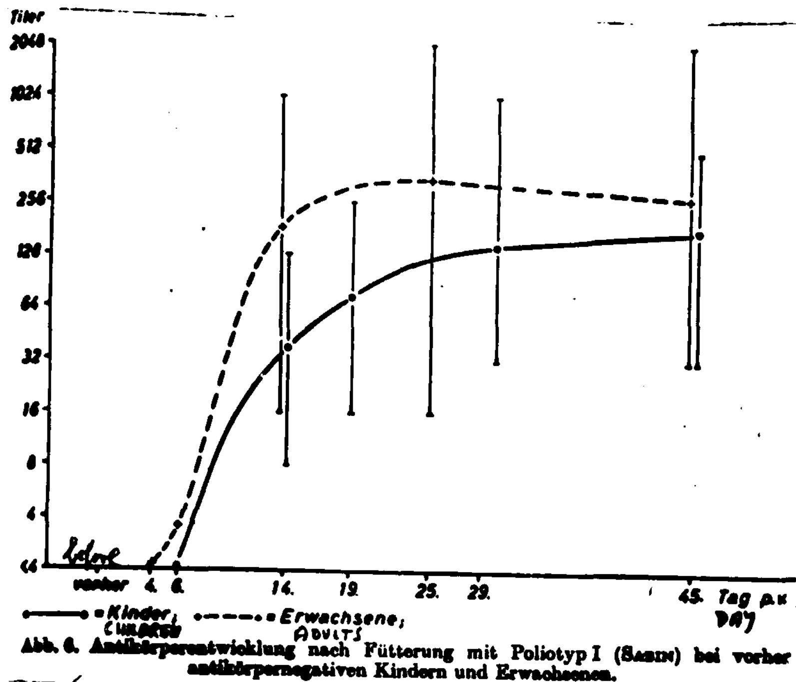

Abb. 6. Antikörperentwicklung nach Fütterung mit Poliotyp I (Sabin) bei vorher antikörpernegativen Kindern und Erwachsenen.

Figure 6. Antibody development after feeding Polio type I (Sabin) in antibody negative children and adults.

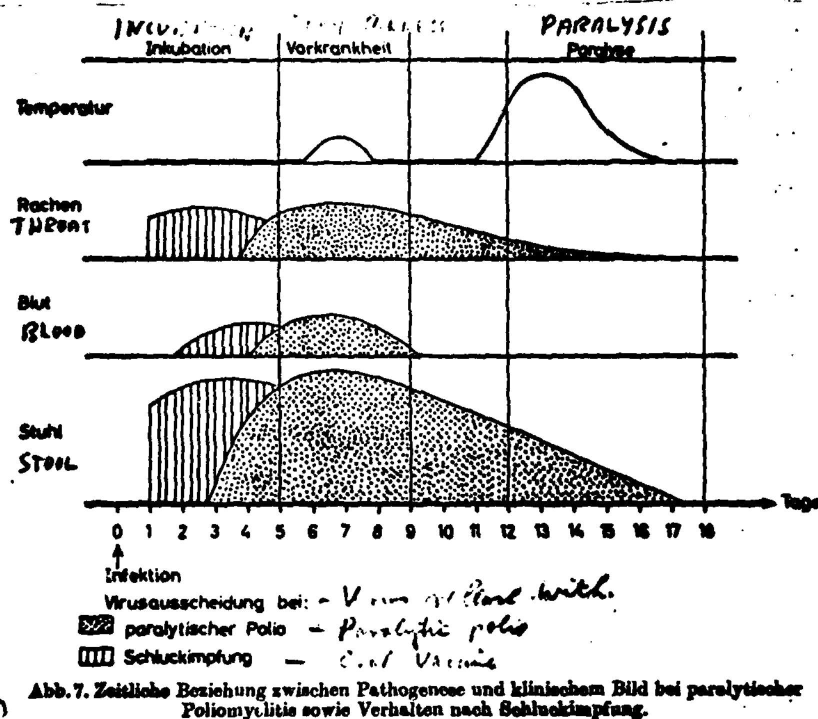

Abb. 7. Zeitliche Beziehung zwischen Pathogenese und klinischem Bild bei paralytischer Poliomyelitis sowie Verhalten nach Schluckimpfung.

Figure 7. Time relationship between pathogenesis and the clinical picture with paralytic poliomyelitis as well as the behavior of the oral vaccine

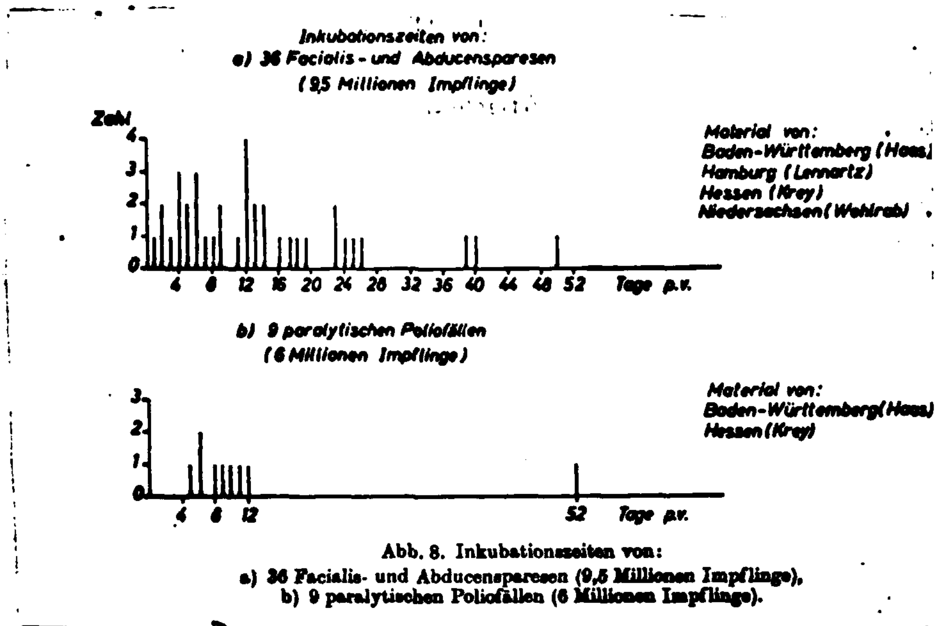

Abb. 8. Inkubationszeiten von:
a) 36 Facialis- und Abducensparesen (9,5 Millionen Impflinge),
b) 9 paralytischen Poliofällen (6 Millionen Impflinge).

Figure 8. Incubation times of: a) 36 facial paralysis and Abducens pareses (9.5 million persons vaccinated); b) 9 paralytic cases of polio (6 million persons vaccinated).

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