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Studies on the Salk and Live Attenuated Poliovirus Vaccine

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SUMMARY

The effect of oral trivalent live attenuated poliovirus vaccine in the chimpanzee was studied and the effect of prior vaccination with the Salk vaccine examined. With the oral vaccine alone, pathological changes compatible with Poliomyelitis were found in the intestinal tract, mesenterial lymphnodes, lungs, liver, spinal cord, brain and choroidal plexus. These changes were prevented by the prior immunization with the Salk vaccine. It is suggested that at the present stage the Salk vaccine should be used together with the oral live virus vaccine.

INTRODUCTION

In a previous paper (Nishizawa *et al.*, 1962) findings with live attenuated poliovirus were reported, and as stated, there are two problems which must be considered with the live poliovirus vaccine. One is the possibility of an increase in neurotropism of the virus. Tests with the Type 1 vaccine of Cox have shown that increase in neurotropism takes place even during a single passage through man. Melnick (1960) has emphasized the same point and Dane (1961) reports that two passages in man of the Types I and II Sabin vaccines resulted in paralysis of the monkey with histological changes compatible with poliomyelitis. Sabin, on the other hand, suggests that though a temporary increase in virulence may occur, a decrease will again take place after 4 or 5 passages. It is believed, however, that even if Sabin's proposal is correct, there is a danger of active infection at the time of increased virulence of the virus. The other problem is the method of assay of virulence. The degree of neurotropism can, of course, be observed by intraspinal or intracerebral injection in the monkey but it should not be forgotten that the poliovirus is viscerotropic as well as neurotropic, that is, it is an enterovirus and proliferates in the intestinal tract or mesenterial lymph nodes. Therefore, it is believed that tests should be by oral administrations since it is an oral vaccine. This is supported by the following findings.

The Sabin oral vaccine was given on a nationwide scale in 1961 and 111 cases

with abnormal symptoms following vaccination were seen in the Pediatrics Department of Osaka University Hospital. The most important were exanthema and polio infection. A rash was noted in 15 per cent of our cases, and according to the national statistics, appeared in 9.5 per cent following Type I vaccine.

The exanthema was urticaria-like and appeared on the day of virus administration or the following day in our cases and usually soon disappeared, though it persisted for a long time in some cases. The abnormal reaction was most severe in a 3 year 11 month old female. In this case, a skin rash became apparent the day after vaccination, but this soon disappeared. On the third day, however, fever, abdominal distension and hepatomegalia occurred. The abdominal swelling and fever gradually increased and she was hospitalized on the 19th day. The Gmelin reaction was weakly positive but jaundice was not seen. A reddish exanthema developed over the whole body and the picture of toxodermia was seen. The patient died with symptoms of reticulosis.

Histopathological investigations were carried out. There were no significant changes in the cervical and thoracic cord and though the majority of the anterior horn cells in the lumbar cord were normal, a few showed swollen cytoplasm, central chromatolysis and disappearance of the nucleus. Slight proliferation of glia was also found. The changes cannot be definitely said to be attributable directly to the live virus vaccine but it cannot be denied that the vaccine may have been a contributing factor.

In several of the cases who developed fever, chest-films showed viral pneumonia. Closer examination may show a greater number of cases with a similar picture and when viral pneumonia occurs following administration of vaccine, it must be considered clinically as polio.

A total of 9 cases of polio following vaccination was hospitalized. The onset of symptoms was 5 days after vaccination in 1 case and this patient died 6 days after admission with signs of Landry's paralysis. Of the remaining 8 cases, poliovirus was isolated in 7 cases and of these, 2 were marker negative with Type I virus in one and Type II in the other.

It cannot be definitely said that the isolated virus is attenuated virus on the basis of the negative marker test but on the other hand, the virus cannot be said to be different even if marker positive.

Melnick (1960) has reported that 5/36 of the virus isolated from the stools of vaccinated children is marker positive and there is a rise in neurotropism in the monkey, especially the Type III virus of Sabin.

Baron *et al.* (1960) from *in vitro* studies found alterations in the property of the marker after heat treatment (Two treatments at 39.5-40.0°C).

It is believed that the latent period in natural infection is 5-10 days but in the case of the live attenuated poliovirus vaccine, it is unknown.

We are not entirely satisfied as to the safety of the Sabin vaccine either from

clinical findings or animal experiments. It cannot be said that the vaccine itself is a direct cause of infection but it is believed that in the presence of some provocative factor, infection may occur.

In an experiment conducted previously by one of the authors, Nishizawa, it was found that polio is induced in the mouse by intravenous injection of a dose of virus which ordinarily will not produce infection, if indian ink is first injected. In other words, there is no affect if the body is healthy but if some defect is present, this increases the sensitivity towards the virus in the vaccine.

Raettig (1961) has studied the effect of the vaccine in West Germany and suggests that infections were due to some provocative factor rather than a direct result of the vaccine. Regardless of the causal factor, it cannot be denied that the cases were infected with polio.

Investigations have been conducted on the relationship between immunization induced by live attenuated and Salk vaccines and a part of the work has been reported previously. In a similar study by the Department of Pediatrics, Tokyo University, (Takatsu, 1962) it was reported that when the oral vaccine was given after 4 doses of Salk vaccine, there was no rise in antibody titer and virus was isolated from the stools in 100 Percent of the cases. From these findings, it is believed that the mode of immunization production with the live and Salk vaccines differs and the Salk vaccine could not prevent intestinal infection but there is a possibility that it prevents viremia induced by live virus vaccine.

MATERIALS AND METHODS

1. *Subjects*

African female chimpanzees captured in Ghana, weighing 4-8 Kg, were used. The animals were tested beforehand to ascertain absence of neutralizing antibodies for poliomyelitis.

2. *Vaccine*

Salk vaccine prepared by Chiba Prefecture Serum Institute was given subcutaneously, in a dose of 1 cc. Sabin vaccine was the trivalent or monovalent preparation of the Connaught Co. of Canada.

3. The vaccines were given according to the method described in Fig. 1 and Fig. 2.

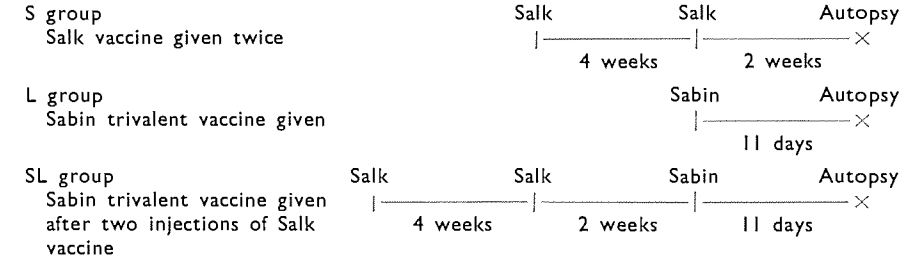

Fig. 1. Conditions in Monkey Experiment

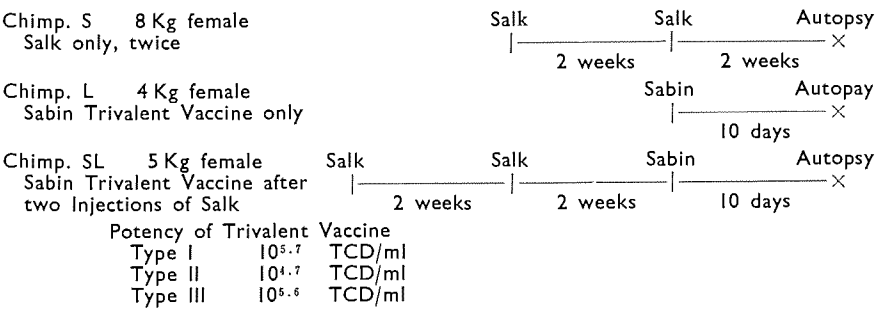

Fig. 2. Conditions in Chimpanzee Experiment

4. *Histological Examination*

The animals were anesthesized with ether and killed by bleeding.

The tissues were stained with hematoxylin and eosin, Nissl's or Bielschowsky's stain.

The tranoscope 6D (Akashi Seisakushyo) electron microscope was used for the electron microscopic examination.

5. *Virus Isolation*

The tissue culture method using the FL cell was utilized for virus isolation and neutralization tests.

RESULTS

Comparative virological and histological studies were conducted. The changes were similar in both the monkey and the chimpanzee but as the changes were more marked in the chimpanzee, these are presented here.

The animal given two injections of Salk vaccine is designated as S, the animal given the Sabin trivalent vaccine alone is designated L and the animal given oral vaccine after two injections of Salk is designated SL.

L was sacrificed 10 days after administration of the vaccine. As can be seen in Tables 1 and 2 ,Type III virus was isolated from the cerebrum, cerebellum and muscle (m. quadriceps femoris) and Type I was isolated from the submandibular lymphode. The isolation of poliovirus from the brain is extremely singificant.

Histological examination of the intestinal tract, revealed the following.

Intestine: The histologic picture of S was within normal limits. In L (Fig. 3), no marked destructive changes were demonstrated in the epithelia lining the mucosa, but marked infiltrations by macrophages and lymphocytes were encountered in the lamina propria and the submucosa. SL exhibited a moderate destructive change of the lining epithelia and marked lymphocytic and moncytic infiltration in the lamina propria and submucosa (Fig. 4).

Mesenterial Lymphnodes: The mesenterial lymphnodes of S were normal. Both L (Fig. 5) and SL (Fig. 6) revealed marked dilatation of the lumen of the sinus, enlargement and desquamation of the endothelia and lymphocytic infiltration. These changes were more marked in L than in SL.

Table 1. Isolation of Virus from Chimpanzee Organs

Conditions of Experiment	Salk only twice	Sabin Trival. Vaccine only	Sabin Trival. Vaccine after two Inections of Salk
Animal / Material	S	L	SL
Cerebrum	—	III	—
Cerebellum	—	III	—
Cervical Cord	—	—	—
Thoracic Cord	—	—	—
Lumbar Cord	—	—	—
Sacral Cord	—	—	—
Heart	—	—	—
Lung	—	—	—
Liver	—	—	—
Bile	—	—	—
Spleen	—	—	—
Kidney	—	—	—
Intestinl Contents	—	I + III	—
Small Intestine	—	—	—
Peyer's Plaque	—	III	—
Large Intestine	—	I + III	—
Mesenterial lymphnode	—	III	—
Submandibular lymphnode.	—	I	—
Muscle	—	III	—

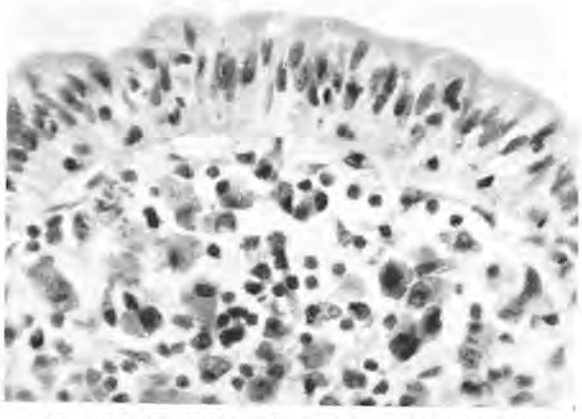

Fig. 3. Small Intestine (Chimpanzee-L) 450X

Table 2. Quantity of Virus Present in Chimpanzee-L

	Day after Vaccination	Type	Quantity (TCD_{50}/gr)
Stool	2	III	$10^{1.5}$
	3	I	$10^{3.5}$
	4	I + III	$10^{3.5}$
	5	I + III	$10^{3.5}$
	6	I + III	$10^{3.5}$
	7	I + III	$10^{3.5}$
	8	I + III	$10^{3.5}$
	9	I + III	$10^{3.5}$
	10	I + III	$10^{3.5}$
Cerebrum		III	$10^{2.5}$
Cerebellum		III	$10^{3.5}$
Intestinal Contents		I + III	$10^{5.5}$
Peyer's Plaque		III	$10^{2.5}$
Mesenterial Lymphnode		III	$10^{2.5}$
Large Intestine		I + III	$10^{3.5}$
Submandibular Lymhnode		I	$10^{4.5}$
Muscle		III	$10^{2.5}$

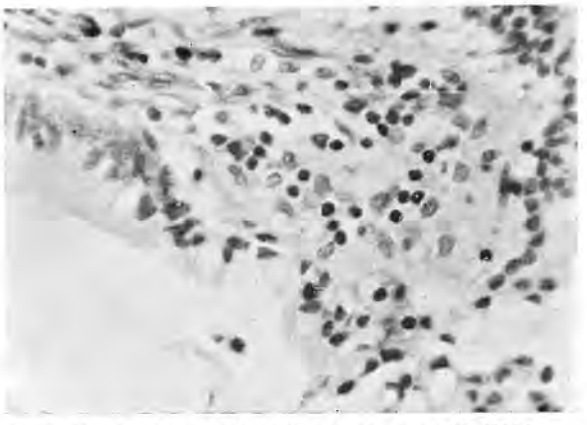

Fig. 4. Small Intestine (Chimpanzee-SL) 450X

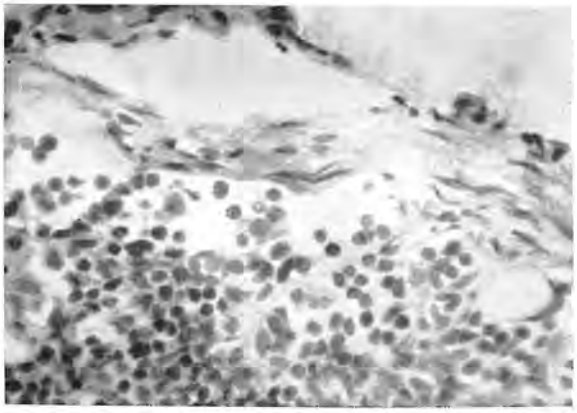

Fig. 5. Mesenterial Lymphnode (Chimpanzee-L) 450X

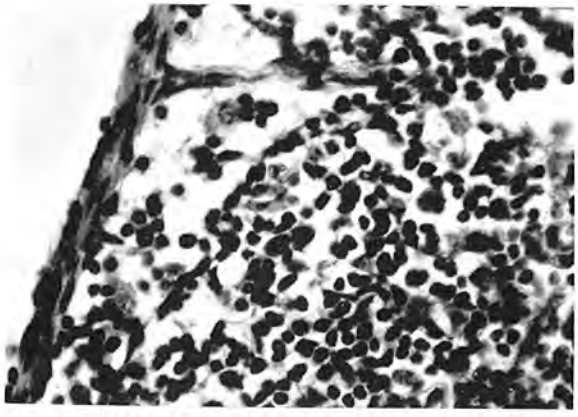

Fig. 6. Mesenterial Lymphnode (Chimpanzee-SL) 450X

Liver: S exhibited moderate peripheral fatty infiltration but otherwise was not remarkable. L (Fig. 7) revealed vascular and central fatty degeneration of liver cells. Enlargement and slight desquamation of Kupffer's cells and a small number of monocytic infiltrations were encountered in the sinus. SL (Fig. 8) demonstrated diffuse fatty infiltration of the liver acini. No degenerative changes were seen in the liver cells.

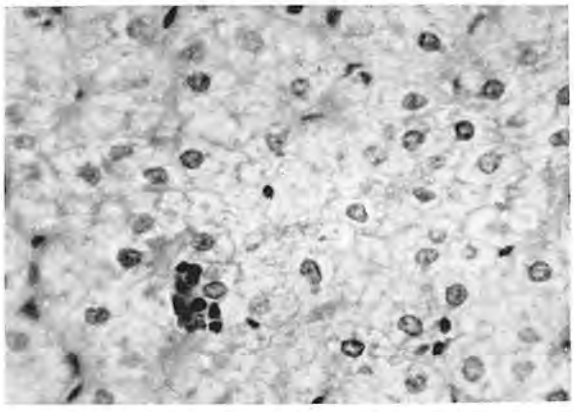

Fig. 7. Liver (Chimpanzee-L) 450X

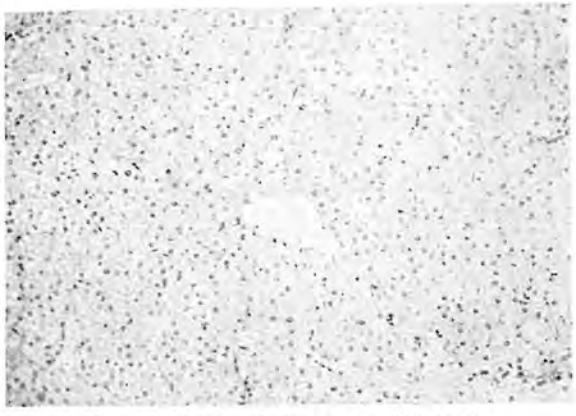

Fig. 8. Liver (Chimpanzee-SL) 100X

Spleen: While S revealed a slight decrease of lymphocytes in the lymph follicles, L (Fig. 9) exhibited marked swelling and degenerative changes of the reticulum cells. These changes, however, were not marked in SL (Fig. 10).

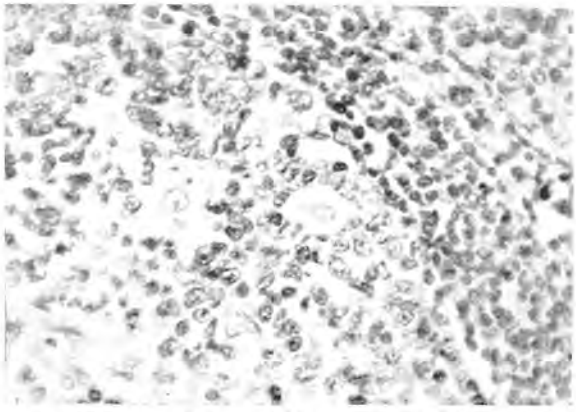

Fig. 9. Spleen (Chimpanzee-L) 450X

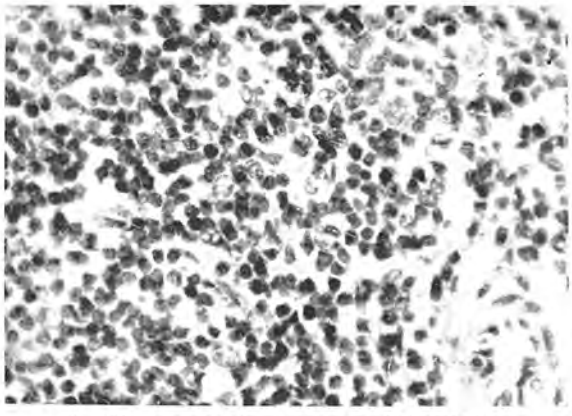

Fig. 10. Spleen (Chimpanzee-SL) 450X

Lungs: Thickening of septum, monocytic and lymphocytic infiltration suggesting interstitial pneumonia were observed in the lungs of L (Fig. 11). No pathological changes were found in S and SL (Fig. 12).

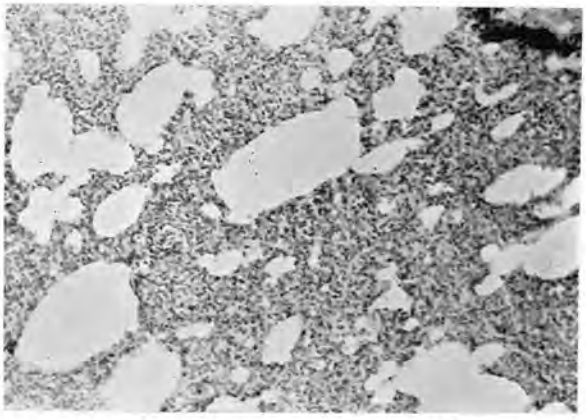

Fig. 11. Lung (Chimpanzee-L) 100X

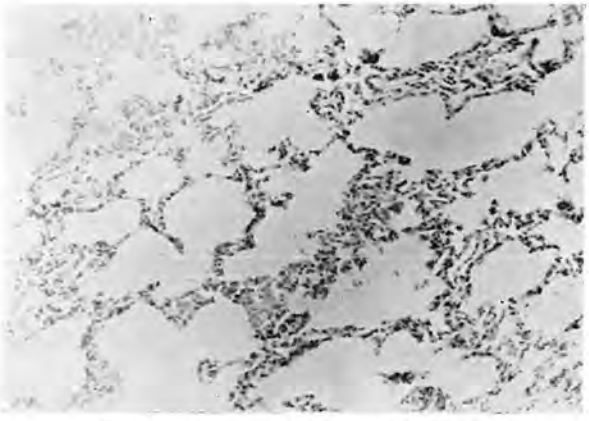

Fig. 12. Lung (Chimpanzee-SL) 100X

Choroidal plexus: While the choroidal plexus of S and SL (Fig. 13) were within normal limits, degenerative changes and desquamation of the lining epithelia and inflammatory cell infiltration in the stromal tissue were demonstrated in L (Fig. 14).

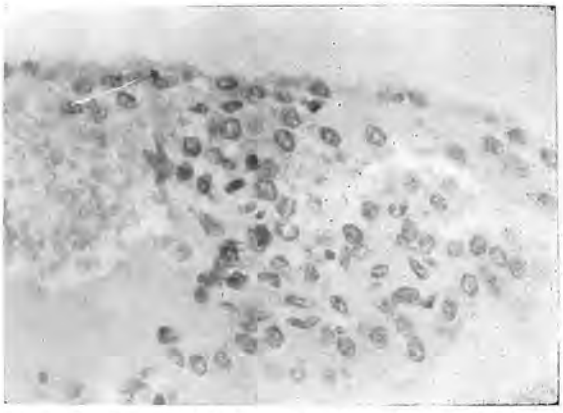

Fig. 13. Choroidal Plexus (Chimpanzee-L) 450X

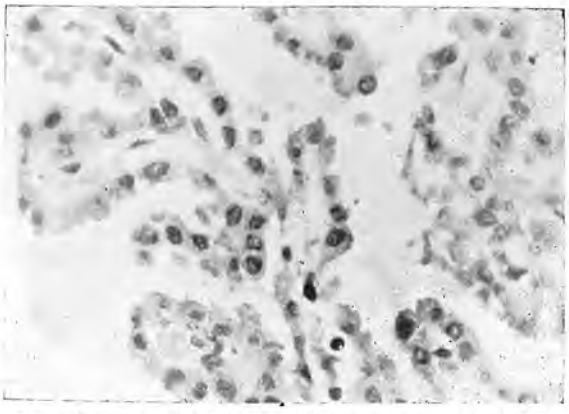

Fig. 14. Choroidal Plexus (Chimpanzee-SL) 450X

Brain and Spinal cord: No marked changes were found in S or SL. In L, marked degenerative changes of nerve cells were often encountered in the anterior central gyrus, midbrain, pons and cerebellum. Fig. 15 shows the changes in the cerebellum from which the poliovirus was isolated. In this picture, degenerative changes and destruction of Purkinje's cells and proliferation of glia cells were found. In the lower portion of the pons, marked congestion and perivascular reaction were observed. In the cranial portion of the cervical cord, atrophy, degenerative change and satellitosis of the motor neurons of the anterior horn (Fig. 16) were encountered.

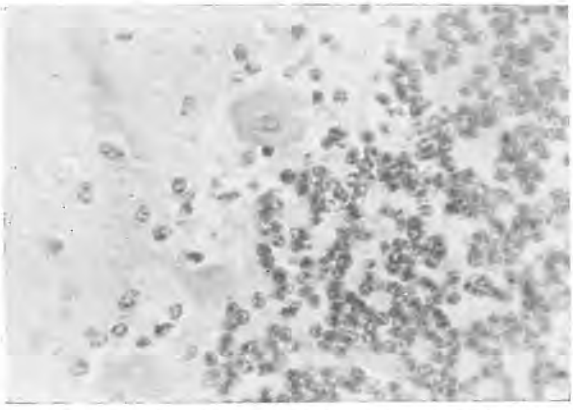

Fig. 15. Cerebellum (Chimpanzee-L) 450X

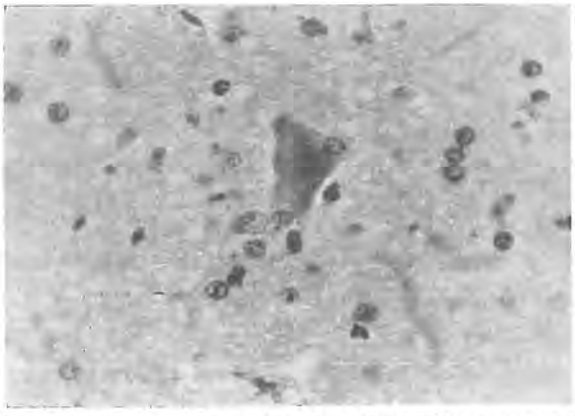

Fig. 16. Cranial Portion of the Cervical Cord (Chimpanzee-L) 450X

An electron microscopic picture of the cervical cord (Fig. 17) showed invasion of 4 glia cells into the degenerative nerve cells.

These changes in the brain and spinal cord were not observed in SL and the Purkinje's cells in the cerebellum were arranged in regular order (Fig. 18).

DISCUSSION

As described above, changes similar to those observed in experimental poliomyelitis in the monkey are found in the intestinal tract, mesenterial lymphnodes, lungs, liver, spinal cord, brain and choroidal plexus following administration of the Sabin trivalent vaccine and in view of the fact that poliovirus was isolated from

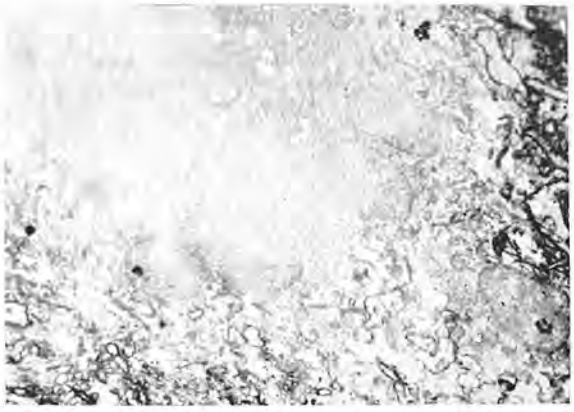

Fig. 17. Cranial Portion of Cervical Cord with Electron Microscope (Chimpanzee-L)

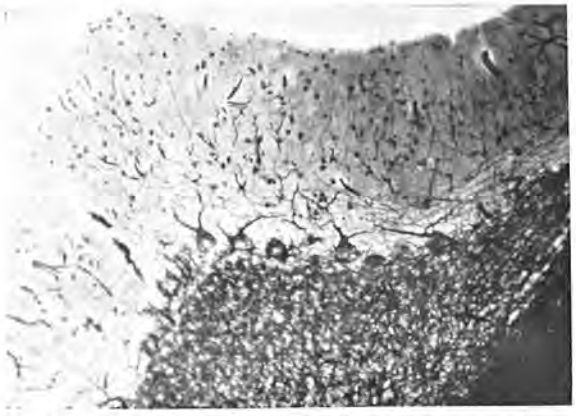

Fig. 18. Cerebellum (Chimpanzee-SL) 100X

these tissues, it must be assumed that these changes are due to the virus it the vaccine. Melnick (1960) has stated that even strains which are considered attenuated still retain some virulence after intraspinal injection but there is no pathological change in the chimpanzee. Our experience with oral administration is contrary to this and the changes observed in the chimpanzee were more severe than in the monkey. The changes in the intestinal tract and mesenterial lymphnodes are understandable in the case of L, but the findings in SL are worthy of note. If immunization is complete with the Salk vaccine, these changes should not have occurred. It is therefore believed that immunization by the Salk vaccine is humoral and though there is a rise in titer of neutralizing antibody in the blood, cellular immunization has not been established. From this, it can be said that a combination of Salk and live virus vaccine is required.

The fact that there were no changes in the liver, lungs, choroidal plexus, brain and spinal cord in SL clearly reveals the immunizing action of the Salk vaccine and this is significant from the standpoint of immunization against polio.

From the investigations using the trivalent vaccine it is suggested that it may not be altogether safe and it may be necessary to change this vaccine. That is, it may only be a specific strain in the trivalent vaccine which is the source of danger.

On the basis of the findings, however, it is believed that at the present stage the Salk should be used together with the live virus vaccine.

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