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PROBLEMS IN THE SANITATION OF MONKEYS FOR WHOLE-BODY IRRADIATION EXPERIMENTS

by

D. van der WAAY and W.M.Th. ZIMMERMAN

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PROCEEDINGS OF THE

INTERNATIONAL SYMPOSIUM ON BONE MARROW

THERAPY AND CHEMICAL PROTECTION

IN IRRADIATED PRIMATES 1962

PROBLEMS IN THE SANITATION OF MONKEYS FOR WHOLE-BODY IRRADIATION EXPERIMENTS

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In previous papers of this group on bone marrow treatment of irradiated monkeys, data were reported on the incidence of various infections with parasites and micro-organisms and their possible significance for the outcome of the experiments. The enhanced susceptibility to infections in irradiated animals and especially radiation chimeras require that every effort should be made to obtain a thorough sanitation of the monkeys before they are used for irradiation experiments.

In this report current procedures for detection and elimination of pathogens will be described as well as their influence on the post-irradiation condition of the animals. Except for a few pig-tail monkeys (Macaca nemestrina) and cynomolgus (Macaca irus) monkeys, our colony consists of rhesus monkeys (Macaca mulatta). Nearly all our information about sanitation problems concerns the latter group. Our last three shipments of rhesus monkeys were supplied according to Tree-to-Laboratory arrangement, which means in practice, that the animals are stocked in India for a few days only before they are flown to Europe. During shipment the animals are kept in individual cages. By reducing the period of exposure to their caretakers in India to an absolute minimum, it is hoped to limit ex-

* This work was performed under contract with Euratom (European Atomic Energy Community) 51-53 rue Belliard, Brussels, Belgium.

posure to human sources of infection. Shortly after arrival the monkeys are put into individual laboratory cages and during the following few days handling is avoided and the animals are disturbed as little as possible. During that initial times feces is collected from the cage pans for bacteriological and parasitological examination. The results of these investigations and data relating to the effectiveness of the current sanitation procedures in preparing the monkeys for radiation experiments will be discussed.

RESULTS

Salmonella and Shigella

As Salmonella and Shigella are the most common causes of enteric bacterial disease in newly imported monkeys, special attention is paid to their detection. This is done by a slightly modified version of the procedure outlined by Galton et al. (1948). The infection usually exists already at the time of arrival of the monkeys, when some of the animals have watery diarrhea. We never saw monkeys die shortly after arrival with hemorrhagic diarrhea, as Fegly and Sauer (1960) and Schneider et al. (1960). From the feces of 198 newly arrived monkeys, Shigella was isolated 55 times and Salmonella 28 times (table I). Because the shipments never con-

NUMBER OF MONKEYS WITH PATHOGENIC INTESTINAL BACTERIA

OUT OF 198 ANIMALS

TIME OF INVESTIGATION		SHIGELLA					SALMONELLA				
		Flexneri II	III	Y	Newcastle	Sonnei	Paratyphi A	B	Bareily	Newport	Typhimurium
At arrival		9	36	5	4	(1)	1	18	1	5	3
Recurrence	1x	1	1	-	1	-	-	1	-	-	-
Recurrence	2x	1	4	-	-	-	-	2	-	-	-
Recurrence	3x	-	2	-	-	-	-	-	-	-	-

Table I

sisted of more than approximately 30 monkeys, individual treatment of the animals was practicable. Antibiotics were preferred to chemotherapy because of their greater effectiveness when administered intramuscularly.

When the feces were positive for Salmonella, the monkeys were treated with chloromycitin for 5 successive days. In case of Shigella infection, treatment with terramycin* was instituted during a similar period. The antibiotics were both administered intramuscularly. In table I the results of the treatment may be evaluated with respect to the number of recurrences. Recently we attempted to decrease this very low incidence of recurrence even further, by testing the sensitivity to a number of antibiotics with test tablets, immediately after isolation of either of these two organisms. If necessary, treatment was then continued with a more effective antibiotic. There are still not enough data on the results of this last procedure to warrant any conclusions. During the period of administration of antibacterial drugs, even when only one or two animals turned out to be infected, the entire colony was kept in quarantaine until negative cultures were obtained from all members of the colony. This measure probably accounts for the fact that spreading of the infection through the colony occurred only rarely. From the feces of the irradiated monkeys that died with hemorrhagic diarrhea, a Salmonella had been isolated in one case only. In two irradiated monkeys Shigella was cultured from one of the daily feces samples.

Intestinal parasites

Among the intestinal parasites, the nematodes are most frequently found in monkeys. Chiefly, three genera of worms may be present, Oesophagostomum, Strongyloides and Trichuris. The members of the second

* A generous gift of terramycin from Pfizer N. V. Nederland is gratefully acknowledged.

genus, represented by a number of species, are more frequently encountered than the others. Only Oesophagostomum however, seems to be of importance to the post-irradiation condition of the host. This is explained by the habit of this worm to burry itself deeply in the intestinal wall. Secondary bacterial infections of the worm lesion may be a fatal complication. The diagnosis of helminth infection was established by the Faust-method of concentration of worm eggs as described by Mackie et al. (1955), while concentration of cysts was carried out by Mansoer's method (1959). Data obtained by these methods are given in table II.

PERCENTAGE OF MONKEYS WITH WORM-EGGS AND/OR CYSTS
(30 MONKEYS)

WORM-EGGS		CYSTS	
Oesophagostomum	88	Entamoeba histolyt.	64
Strongyloides	91	Entamoeba Polecki	8
Trichuris	75	Entamoeba Hartm.	12
Unidentified	3	Iodamoeba Buetschlii	7
		Endolimax nana	44
		Lamblia intest.	3
		Blastocystis	51

Table II

Nearly 10% of the irradiated monkeys showed multiple nodules, harbouring Oesophagostomum larvae, in the wall of the large intestine, the omemtum, and mesenteries and less frequently in the stomach, abdominal wall and liver. Secondary bacterial infection of these nodules apparently was the cause of the local or general peritonitis, which was observed in several cases. The lesions described are characteristic for this worm. The pre-adult larvae obtained from the nodules of two monkeys were classified as Oesophagostomum apiostomum by Prof. P. H. van Thiel.*

* From the Department of Parasitology, Institute of Tropical Medicine, Leyden, The Netherlands.

The Oesophagostomum eggs pass in the feces where, under suitable conditions the development into an infectious larval stage occurs in the course of about 5-7 days. It is assumed that the mode of entry of the larvae is by way of the mouth, although it is stated by Graham (1960) that the possibility of a percutaneous route of entry in primates should not be excluded in view of the findings of Mayhew (1939) in calves. The ensheated third-stage worm enters the wall of the cecum and colon, where nodule formation occurs (figure 1). Inside the nodule the worm passes through its third

Figure 1

Noduli in the wall of the colon of Macaca mulatta, caused by Oesophagostomum apiostomum.

ecdysis and loses its cuticle, to become a pre-adult worm. This fourth-stage worm usually re-enters the lumen of the intestine, but when the host is highly immune, it appears to be permanently trapped in the nodule. (Graham, 1960). As a rule the adult worms do not attach themselves to the wall of the colon to suck blood like the Ankylostomum species. The period during which the larvae reside in the intestinal wall is still uncertain, but it is believed to be some seven days in non-immune animals (Graham, 1960 and Ruch, 1959). To establish a possible relation between the mean number of nematode eggs per gram of feces and the number of nodules in the intestinal wall, 5 separate portions of feces of each monkey that arrived with the last shipment, were investigated. As is shown in table III, no relation was demonstrable between the average number of Oesophagostomum eggs found per gram of feces and the number of nodules found at autopsy some weeks later. An additional purpose of the investigation was to get an impression of the severity and incidence of Oesophagostomum infection with nodule formation among newly arrived animals, in comparison to animals that have been

THE RELATION BETWEEN THE MEAN NUMBER OF OESOPHAGOSTOMUM-EGGS PER GRAM FECES OF FIVE SEPARATE PORTIONS IN NEWLY ARRIVED MONKEYS

Incidence of nodules in the wall of the colon	Mean number of eggs
++	10.7
++	8.5
++	4.3
+	4.0
++	3.9
+	2.5
++	2.5
++	2.3
++	2.0
-	1.9
++	1.8
+	1.8
-	1.8
+	1.2
+++	1.0
++	1.0
-	1.0

Table III

under laboratory conditions for some time. As compared to the frequency of nodules in animals that have been under laboratory conditions for 6 months or more, the incidence shown in table III is rather high. The possibility exists that the number of nodules has a tendency to decrease in the course of time when the monkeys are kept in strict isolation and when measures are instituted to prevent re-infection. However, a more systematic investigation has to be performed in order to prove this hypothesis.

In our opinion the detection of Oesophagostomum eggs in the feces of newly arrived monkeys, probably is only of value if their mean number is compared with similar egg counts at a later date. An increase of eggs at that time, could indicate that a massive infection with many nodules had been present initially and that the larvae of the nodules have grown into adult worms between both observations.

An attempt has been started to destroy the adult worm with the new antihelmintic drug Alcopar (Bephenium Hydroxynaphtoate), in a group of 28 monkeys. After an initial egg count was performed in the concentrate of one gram of feces, the drug was given orally in amounts of 2 gm/kg body-weight following a fast period of 24 hours. The egg count was repeated every three weeks during the following 4 months. Twelve weeks after the first treatment with Alcopar, a second similar dose was given to those monkeys that still had Oesophagostomum eggs in their feces. In figure 2 the mean number of worm eggs in the feces of all 28 monkeys is plotted against the time of investigation. The effect of the drug on adult Oesophagostomum in the intestinal lumen seems to be rather favourable. The fact that Strongyloides usually embryonate very early (in some cases the larvae may even develop in the last part of the colon) may explain why Strongyloides eggs do return in great numbers rather soon after the administration of Alcopar. To eliminate Strongyloides infection the drug should perhaps be administered more frequently.

Protozoal intestinal infections

Infection with intestinal protozoa may have contributed to the death of two irradiated monkeys. In these animals Entamoeba hystolitica was demonstrable in colonic ulcers. The frequency of histolityca cysts in the feces

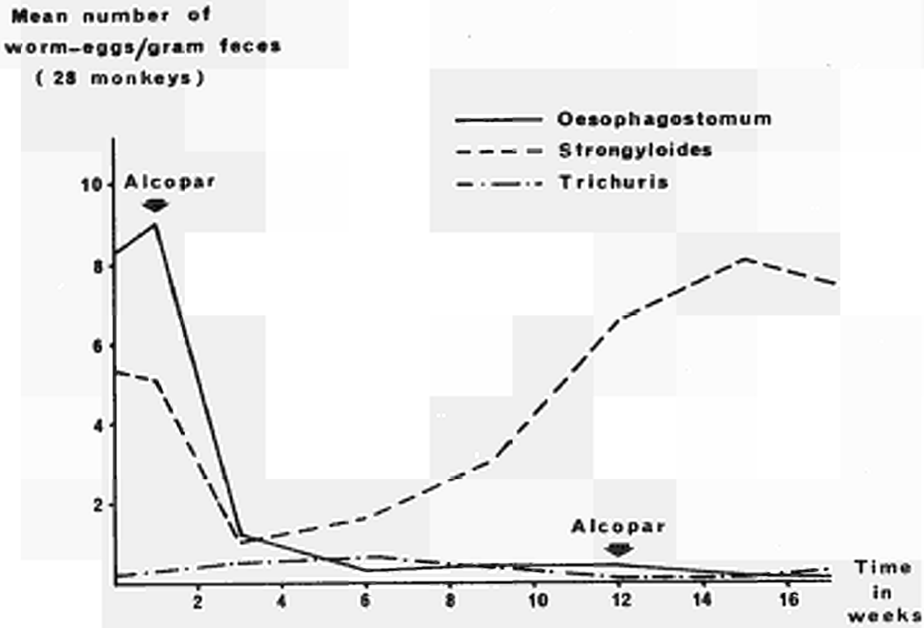

Figure 2

Mean number of worm-eggs per gram feces of 28 monkeys as influenced by treatment with Alcopar.

of our monkeys (table II) is rather high in comparison to data reported by Ruch (1959), but since invasiveness of the Entamoeba was so rarely found in the absence of specific treatment, it is not believed that this parasite is of great significance. It has been suggested by Ruch (1959) that Entamoeba histolytica is of minor importance, either because it is less pathogenic for monkeys or because the monkey has a stronger resistance against this infection than man.

Tuberculosis

Three monkeys died with widely dissiminated pulmonary tuberculosis, which unfortunately was not diagnosed until the monkey was autopsied. A few days after arrival, each animal was tested intracutaneously with a dilution of 1:10 of Koch's old tuberculin in the right eyelid. The dose administered is equivalent to 10 mg which dose is also being used by Young et al. (1957). This procedure was repeated monthly during the first three months after arrival and thereafter quarterly. The tuberculin solution used in these skin-tests was checked afterwards in two tuberculous guinea pigs, and this resulted in positive reactions in dilutions of 1:100 and 1:1000. When the first case of tuberculosis was discovered at autopsy, the Mantoux-test was repeated immediately on the monkeys in the same room and turned out to be negative in all cases. It should be noted that in the three tuberculous monkeys the Mantoux-tests had also been negative. The first two affected animals were rather ill during the last week before they died. The last one on the contrary, showed no loss of weight but was coughing, probably as a result of pressure on the trachea by an enlarged para-tracheal lymphatic gland. The second and the third case were members of the same shipment and for that reason the whole group was killed. At autopsy no suspicious foci were found in these animals. In smears of the spleen and lung stained by the Ziehl-Neelson method no acid-fast rods were demonstrable.

The results show that the Mantoux-test in our hands is a highly unreliable index of tuberculosis infection in rhesus monkeys. On the other hand the contagiousness of the disease is apparently very low; although the infected monkeys had been coughing for several days and even for several weeks in the last case, all neighbouring monkeys proved to be negative at necropsy. These findings differ from those published by Young et al. (1957) and Gisler et al. (1960) who obtained satisfactory results by using the Mantoux-test and also reported a rapid spread of the tuberculous infection in their monkey colony, if they had an infection at all.

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