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WORLD HEALTH ORGANIZATION
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No. 74

EXPERT COMMITTEE ON
PLAGUE

Second Report

	Page
1. Election of Chairman	3
2. Adoption of the agenda	3
3. Wild-rodent surveys	3
4. Methods of differentiation between plague and pseudo-tuberculosis bacilli, and possibilities for the standardization of these procedures	5
5. Biochemical reactions of <i>P. pestis</i>	5
6. Recent studies of immunity response to administration of different plague vaccines	6
7. Treatment and prophylaxis of plague by drugs	6
8. Evaluation of modern rodenticides, with special reference to anticoagulants	8
9. Recent observations of plague control with DDT, BHC, and calcium cyanide dust	9
10. Discussion of a memorandum from the Director-General to the Expert Advisory Panel on Plague regarding the plague provisions in the International Sanitary Regulations	10
Annex 1. Basic methods for the laboratory diagnosis of plague	11

WORLD HEALTH ORGANIZATION

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OCTOBER 1953

EXPERT COMMITTEE ON PLAGUE

Second Session

Bombay, 5-10 December 1952

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The report on the second session of this committee was originally issued in mimeographed form as document WHO/Plague/29, 9 April 1953.

EXPERT COMMITTEE ON PLAGUE

Second Report¹

The second session of the Expert Committee on Plague was held at the Haffkine Institute, Bombay, from 5 to 10 December 1952.

1. Election of Chairman

Sir Sahib Singh Sokhey was unanimously elected Chairman. Dr. G. Girard was elected Vice-Chairman, and Dr. A. Castro, Rapporteur.

2. Adoption of the Agenda

The provisional agenda was adopted without alteration.

3. Wild-Rodent Surveys

3.1 *Results of African survey*

The committee gave consideration to a report on wild-rodent plague in Africa² based on an analysis of replies to a questionnaire distributed to the governments of African territories, including Madagascar, following a recommendation made at the first meeting of the committee in September 1949.³

During the period covered by the inquiry (1935-49), a marked decline in the annual incidence of human plague took place, particularly from 1946 onwards. In 1949, less than 400 cases were reported, as against more than 6,000 in 1935. Nevertheless, by the end of 1949, plague was

¹ The Executive Board, at its twelfth session, adopted the following resolution:
The Executive Board

1. NOTES the second report of the Expert Committee on Plague;
2. THANKS the members of the committee for their work; and
3. AUTHORIZES the publication of the report.

(Resolution EB12.R12, *Off. Rec. Wld Hlth Org.* 49, 4)

² Davis, D. H. S. (1953) *Bull. Wld Hlth Org.* 9 (in press)

³ *Wld Hlth Org. techn. Rep. Ser.* 1950, 11, 6

still active in southern Africa, the Belgian Congo, Kenya, Madagascar, and Tanganyika, where conditions evidently favour the persistence of the infection.

In southern Africa, the permanent reservoir is maintained by certain wild rodents, whereas in the Central African highlands and Madagascar, domestic rodents seem to be the main reservoir. Although wild rodents have been found infected in Central Africa, their importance in maintaining the infection has not been fully assessed. In Madagascar, no evidence has been found so far that wild rodents are significantly concerned.

3.2 Possibilities of surveys in other territories

The committee also noted a document on plague in Argentina,⁴ where, apart from sporadic human cases, the infection is confined at present to wild rodents of the hinterland.

The committee was informed that the initial results of a long-term rodent survey in Brazil had not revealed any plague infection in wild rodents. Domestic rodents seemed to be the sole reservoir of the infection.

Reference was also made to recent findings of scattered foci of wild-rodent plague in Iranian Kurdistan and in Turkey, near the Syrian border; the opinion was expressed that these foci merely formed a part of a much wider plague enzootic area.

The committee noted that, because foci of wild-rodent plague were, as a rule, situated in sparsely populated semi-desert areas and wild-rodent fleas did not normally have the opportunity of biting man, human infections in such foci were rare. However, a dangerous situation was apt to develop if wild rodents passed the infection to domestic rodents in or around human settlements. The unexpected occurrence of human plague in insufficiently explored areas draws attention to the need for an exact delimitation of wild-rodent plague.

Taking into consideration that the relationship between wild-rodent and domestic-rodent plague has not been fully elucidated, it was further recommended that countries in which plague is still endemic should undertake systematic studies on ecological lines to establish the comparative importance of wild- and domestic-rodent species in the maintenance of the plague reservoir, and that particular attention should be given to a comprehensive investigation of the vector ability and survival of plague-infected fleas under different conditions of temperature and humidity, both in the laboratory and in the field.

⁴ Barrera, J. M. de la (1953) *Bull. Wld Hlth Org.* 9 (in press)

4. Methods of Differentiation between Plague and Pseudotuberculosis Bacilli, and Possibilities for the Standardization of These Procedures

The committee noted a document, dealing with the differentiation of *Pasteurella pestis* and *P. pseudotuberculosis*,⁵ wherein it was stated that, for epidemiological and clinical purposes in everyday laboratory work, these two micro-organisms could be easily differentiated. The motility of the pseudotuberculosis bacillus, its lack of pathogenicity when inoculated subcutaneously into white rats, the production of urease, and the constant fermentation of glycerol and rhamnose usually suffice to distinguish it from *P. pestis*.

Serological tests (agglutination and precipitation) and tests with bacteriophage strains, if carried out in a suitable manner, give fully reliable results and should therefore be used for differential-diagnostic purposes in the case of doubtful strains, in addition to the combined use of several of the above-mentioned, simpler procedures.

The committee realized the difficulty of using, universally, tests with diagnostic sera and specific phages for a differentiation of the two micro-organisms, but stated that these difficulties might be obviated if one or a few key laboratories could be entrusted with the preparation and distribution of suitable sera and bacteriophage strains. It was noted in this connexion that, while a differentiation of plague from pseudotuberculosis bacilli was of practical importance in some areas, particularly in France where human infections with the latter micro-organisms were not rare, *P. pseudotuberculosis* had so far not been found by plague workers in Central Africa, China, India, Indo-China, and Madagascar.

5. Biochemical Reactions of *P. Pestis*

Considering a paper summarizing present knowledge on the classification of *P. pestis* by biochemical methods and suggestions for further studies of the biochemical reactions of *P. pestis*,⁶ the committee decided that, in view of its great importance in elucidating questions of epidemiology, the glycerol fermentation test was essential in the bacteriological diagnosis of plague. Key laboratories, using a mutually standardized procedure of testing, should be named to facilitate this work. The great stability of the negative reactions produced by a considerable part of the plague strains in glycerol media appears to be significant. Further work along these lines should be conducted systematically.

⁵ Girard, G. (1953) *Bull. Wld Hlth Org.* 9 (in press)

⁶ Pollitzer, R. (1953) *Bull. Wld Hlth Org.* 9 (in press)

The phenomenon of halo production round *P. pestis* colonies on blood agar deserves, in the opinion of the committee, study conjointly with glycerol tests.

The nitrate reduction test and tests demonstrating the production of nitrous acid in nitrate-free media were considered important in the light of newer knowledge, but a further study of these reactions under standardized conditions would be desirable before any final conclusions concerning their reliability and usefulness in the determination of subvarieties of the plague bacillus could be made.

The committee appointed a subcommittee to work out basic standardized methods for the laboratory diagnosis of plague (see Annex 1, page 11).

6. Recent Studies of Immunity Response to Administration of Different Plague Vaccines

Experimental evidence⁷ presented to the committee indicated that any plague vaccine, whether live or killed, was capable of altering the susceptibility of the inoculated, provided that the vaccine used contained an adequate amount of immunizing material. Killed vaccines should be administered in two doses.

Annual revaccination with a single dose progressively improves the immune status of the population groups and thus serves as an effective means in the control of plague in endemic areas.

With live vaccines local reactions are essential, while with killed vaccines satisfactory protection can be obtained with preparations producing neither local nor general reactions.

7. Treatment and Prophylaxis of Plague by Drugs

7.1 Relative value of sulfonamides and antibiotics

From a document presented to the committee, it was noted that recent trials to cure bubonic-plague patients in India had given the following results:⁸

Drug	All cases			Cases with septicaemia at the commencement of treatment		
	Number of cases	Number of deaths	Mortality (%)	Number of cases	Number of deaths	Mortality (%)
Streptomycin	148	6	4.2	37	4	10.8
Sulfadiazine	180	16	8.9	62	13	21.0
Sulfamerazine	113	9	8.0	22	7	31.8

⁷ Meyer, K. F. (1953) *Bull. Wld Hlth Org.* 9 (in press)

⁸ Sokhey, S. S., Wagle, P. M. & Habbu, M. K. (1953) *Bull. Wld Hlth Org.* 9 (in press)

Streptomycin gave, therefore, markedly better results than sulfadiazine and sulfamerazine.

The committee was further informed that streptomycin also proved most satisfactory in the treatment of pneumonic-plague cases in Madagascar. It was noteworthy that, although plague bacilli continued to be present in the sputum of these patients, they proved no longer virulent 24 hours after the commencement of treatment.

While, as shown by the above figures, sulfonamides have given only mediocre results in the patients showing secondary septicaemia at the commencement of treatment, it was noted that these drugs have proved more satisfactory when administered before septicaemia developed. Similarly, the committee was informed that, in Brazil, where a mild type of plague prevailed, only 6% of 600 bubonic-plague patients who had been treated with a mixture of sulfathiazole, sulfadiazine, and sulfamerazine succumbed to the disease. It followed that, in order to be effective, treatment with sulfonamides alone had to be started early in the disease before septicaemia developed. This is a point of great importance for those countries where, for fiscal reasons, it is impossible to make large-scale use of antibiotics for the treatment of plague.

Discussing the problem of plague prophylaxis with therapeutic substances, the committee noted that immune serum, although giving occasional good results in the past, could never be used on a large scale. Chemoprophylaxis with sulfonamides which could be universally used has given excellent results, for instance, in Madagascar, where, since the introduction of this method 12 years ago, no instances of manifest infection have been observed in the contacts of pneumonic-plague patients.

While chemoprophylaxis with sulfonamides is most essential for the protection of contacts of pneumonic-plague patients, it should also be used, under exceptional conditions, for individuals exposed to bubonic infection, for instance, to afford temporary protection to staff who have to enter plague-infected localities soon after they have been vaccinated against plague.

Sulfamerazine, because of its less rapid disappearance from the blood than other sulfonamides, appears to be particularly suitable for the purposes of chemoprophylaxis.

7.2 Standardization of treatment and prophylactic methods

In the experience of the members of the committee, the treatment of pneumonic plague should be carried out with streptomycin in a dosage of about 16-20 g administered during a period of from six to seven days. The same treatment should be applied to severe cases of bubonic plague

with septicaemia. It was further recommended that this form of treatment should be supplemented by suitable supportive treatment. In pneumonic plague, the administration of sulfonamides or wide-spectrum antibiotics might be necessary in order to prevent secondary infections or their sequelae.

Early cases of bubonic plague can be effectively treated with sulfonamides using a dosage of about 10 g on the first day, followed by smaller doses, making a total of at least 50 g during the first week. The first administration of sulfonamides in the dose of 2 g should be made intravenously, preferably in a glucose solution. In order to prevent relapses, particularly meningial plague, treatment with sulfonamides should be continued for at least three days after the temperature becomes normal.

For the protection of contacts to pneumonic-plague patients, the committee recommended the administration of daily doses of 3 g of a suitable sulfadrag over a period of six days. The committee likewise recognized the value of prophylactic vaccine after this course of treatment.

8. Evaluation of Modern Rodenticides, with Special Reference to Anticoagulants

After a thorough discussion of the possibilities of using rat poisons for the purposes of plague control, the committee reached the opinion that, during outbreaks, the use of insecticides was the essential method of control. The destruction of rats was also an important measure, especially during the interepidemic periods. The routine use of insecticides should be continued during these periods.

Discussing the various rodenticides,⁹ the committee drew attention to the value of the recently introduced anticoagulants. These substances kill the rodents gradually and do not render them poison-shy.

The committee agreed that, of the poisons recommended at the first meeting,¹⁰ sodium fluoracetate (1080) was by far the most effective. However, while it is therefore desirable to use this compound whenever possible, it should be used solely under conditions where the accidental poisoning of human beings and domestic animals can be effectively prevented.

ANTU (α -naphthylthiourea), red squill, and zinc phosphide, which were discussed at the first session of the committee, were considered less effective than 1080. However, the committee was of the opinion that they could be used, with due precautions, in warrantable conditions. The committee was definitely against the use of virus preparations as rodenticides.

⁹ Link, V. B. (1953) *Bull. Wld Hlth Org.* 9 (in press)

¹⁰ *Wld Hlth Org. techn. Rep. Ser.* 1950, 11, 13

9. Recent Observations of Plague Control with DDT, BHC, and Calcium Cyanide Dust

As was noted from a report¹¹ submitted to the committee, recent comparative studies on the pulicidal value of DDT, BHC, and calcium cyanide dust undertaken in India have shown that, for the purposes of flea control, the first-mentioned insecticide was most effective, while BHC was less effective, and calcium cyanide dust least effective.

Field observations made in the course of these studies have shown that by applying DDT residual spray inside all the houses of a plague-infected village—and preferably, also, DDT dust to the rat burrows in and around the houses—the spread of the infection could be stopped completely in about 8-10 days in the majority of instances.

Considering these and other observations, the committee recommended the combined application of DDT to houses and to rat burrows as the method of choice in plague control.

It deserves great attention that recent field observations in Ecuador¹² reported to the committee have furnished proof that repeated applications of DDT can render rat-flea populations resistant to this insecticide. Since it would not be fully satisfactory to use BHC in place of DDT under such conditions, it was, in the opinion of the committee, essential to search for new insecticides which were as effective against fleas as DDT.

The committee expressed the view that the application of calcium cyanide dust for the purposes of plague control should not be encouraged.

Summarizing their discussions on the control of plague, the committee laid stress upon the fact that, in view of the marked differences existing between different plague areas in regard to the character and intensity of the manifestations of the disease, the conditions under which the people lived, as well as the funds and facilities available for campaigns, it was impossible to draw up one rigid scheme of plague control. Programmes adapted to the local conditions should be implemented in each area. The committee emphasized, however, that under all circumstances the ideal method of fighting plague was to cut short the contact between rodents and man through the improvement of houses.

The authorities ought to be urged, therefore, to spare no effort to improve the housing of the people in the plague-affected areas as quickly as possible. The authorities should also maintain an efficient antiplague organization, the most essential aim of which should be to detect incipient manifestations of the infection, and to deal with them as they arise. Adequate suppressive measures should be carried out all the time.

¹¹ Wagle, P. M. & Seal, S. C. (1953) *Bull. Wld Hlth Org.* 9 (in press)

¹² Sáenz Vera, C. (1953) *Bull. Wld Hlth Org.* 9 (in press)

10. Discussion of a Memorandum from the Director-General to the Expert Advisory Panel on Plague Regarding the Plague Provisions in the International Sanitary Regulations

In accordance with a resolution of the Executive Board at its eighth session,¹³ a memorandum¹⁴ on the provisions relating to plague in the International Sanitary Regulations (World Health Organization Regulations No. 2)¹⁵ was presented to the committee for comment and recommendations. In this document it was pointed out that, in framing these regulations, a compromise on certain points had to be reached and that, for this reason, the text did not always attain the ideal of technical perfection. It was recommended that any technical criticisms of, or proposed amendments to, the Regulations should be made in the full knowledge of the difficulties encountered in reaching an agreement regarding some points.

Bearing these considerations in mind, the committee did not wish to make recommendations.

¹³ Resolution EB8.R13, *Off. Rec. Wld Hlth Org.* 36, 4

¹⁴ Unpublished working document WHO/Plague/18

¹⁵ *Wld Hlth Org. techn. Rep. Ser.* 1951, 41

Annex 1**BASIC METHODS FOR THE LABORATORY DIAGNOSIS
OF PLAGUE****1. Human Material**1.1 *Venous blood*

Volume of sample : 5-10 ml.

1.1.1 Smear : One drop on slide. Stain with polychrome methylene blue or a stain of the Romanowsky type.

1.1.2 Blood culture

1.1.2.1 0.25 ml on each of two agar slopes (pH 7.2). Incubation at 25°-28°C.

1.1.2.2 4.5 ml in peptone water (pH 7.2). Incubation at 25°-28°C.

1.1.3 Animal experiments : Direct subcutaneous inoculation of 5.0 ml into a guinea-pig or of 0.5 ml into a white mouse.

1.1.4 Further procedures

1.1.4.1 Agar slopes :

- (1) Examine with hand lens after 24 and 48 hours.
- (2) Make smears from sticky colonies and stain with Gram and methylene blue with a few drops of carbol fuchsin.
- (3) Subinoculate material from sticky colonies into
 - (a) peptone water—two tubes for Indol test and tests for the presence of nitrites respectively ;¹
 - (b) fermentation tubes with glucose, glycerol, rhamnose, and sucrose ;
 - (c) blood agar slant (for smears and urease test) ;
 - (d) semi-solid agar (0.4 % - 0.5%) for motility test.

1.1.4.2 Peptone water :

If growth produces no uniform turbidity after 48 hours, plague may be suspected. Make subculture on agar slant and carry out tests recommended in section 1.1.4.1.

¹ Trials should be made to establish whether casein hydrolysate media are suitable for nitrate reduction tests.

NOTE : If agglutination tests are indicated, a peptone water culture with 0.2% formalin should be used as antigen.

1.1.4.3 Animal experiments :

(1) Guinea-pig : Observe development of bubo. On fourth or fifth day, make heart puncture and examine blood as specified in section 1.1. If inoculated with virulent material, the animal should die within 5-6 days. If animal does not die, sacrifice it after 15 days.

At autopsy search for macroscopic signs of plague and make smears and cultures from any bubo present, heart-blood, liver, spleen, lungs, and, if necessary, also from bone-marrow.

(2) White mouse : If inoculated with virulent material, the animal should die in 2-4 days. If animal does not die, sacrifice it after 10 days.

Follow autopsy procedures specified in the case of the guinea-pig. If doubtful findings are made, an inoculation into a guinea-pig is recommended.

NOTE : If the mouse shows signs of decomposition, gentian-violet agar should be used for cultivation.

1.2 *Material from buboes*

Single puncture, without rotation, made with a fine needle (20 gauge or 0.7 mm), and collection in saline solution or peptone water already sucked into the syringe (a few drops) are recommended.

Smears and cultures should be made and examined as specified in sections 1.1.1, 1.1.2, and 1.1.4.

1.3 *Bone-marrow*

Bone-marrow should be obtained post mortem only through sternal puncture or digitomy.

Cultures and animal experiments should be made as specified in section 1.1.

NOTE : In the case of decomposed corpses, it is recommended that complement-fixation tests should be made with bone-marrow or spleen material, according to the method described by Chen et al.²

1.4 *Sputum*

1.4.1 Smears should be made and stained as specified in section 1.1.1. Examinations are to be repeated at intervals of a few hours if no typical results are obtained at first.

² Chen, T. H., Quan, S. F. & Meyer, K. F. (1952) *J. Immunol.* **68**, 147

1.4.2 Guinea-pig inoculation by scarification.

1.4.3 Cultivation may be attempted on agar containing 500 units penicillin per ml.

1.5 *Cerebrospinal fluid*

A sample of 5 ml should be taken if plague meningitis is suspected. Cultivation as specified in section 1.1.2.

1.6 *Blood serum*

1.6.1 Agglutination with repeatedly washed antigens.

1.6.2 Complement-fixation tests according to the method of Chen et al.

2. Animal Material

2.1 *Live animals sacrificed in the laboratory*

2.1.1 Post-mortem examination with special attention to the lymph-nodes, liver, spleen, and lungs.

2.1.2 Smears from these organs as specified in section 1.1.1.

2.1.3 Animal inoculation with material from these organs as recommended in section 1.1.3.

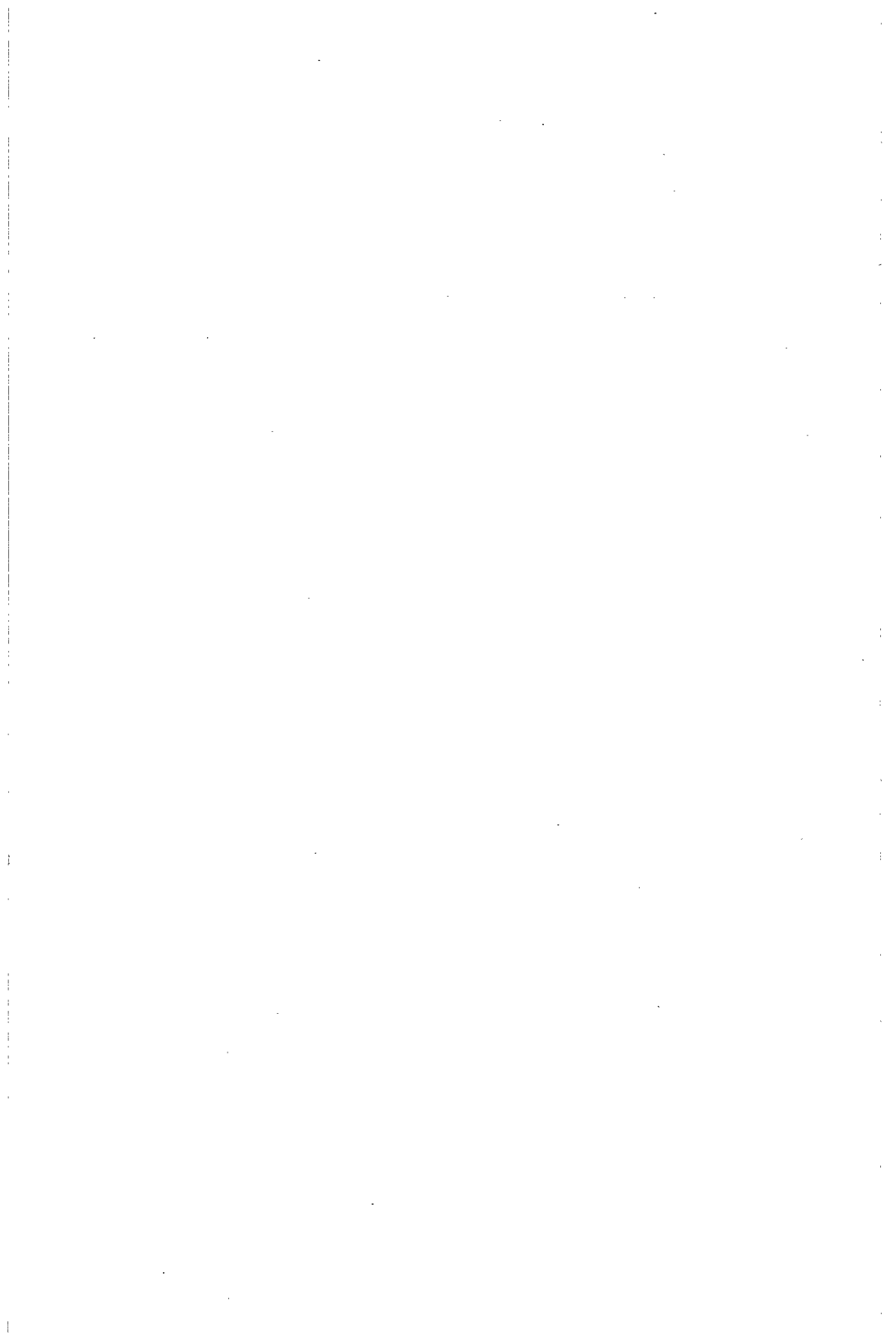
2.2 *Dead animals*

Post-mortem examination as recommended in section 2.1.1, followed, if the carcasses are fresh, by smear examination and inoculation of test-animals.

If the internal organs are decomposed, the bone-marrow should be examined as specified in section 1.3.

3. Fleas

Subcutaneous inoculation of a guinea-pig with fleas crushed in a small quantity of normal saline solution, using pools not exceeding 100 (preferably, 20-30) specimens for this purpose.



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