

A CONTRIBUTION TO THE VIRUS THEORY OF CANCER

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In October 1950 I published in this *Journal* an article under the heading *Primary Carcinoma of the Liver in East African Natives: is it caused by an Infective Agent*¹? Peculiar inclusion-bodies were found in cells of the anterior lobes of the hypophyses of 17 Native mine workers who died of primary carcinoma of the liver during the years 1929-39, and which were produced in the adrenals of guinea-pigs inoculated with emulsions of the affected hypophyses. The role the hypophysis and the other endocrinal glands may play in contributing to the formation of malignant growths was discussed. The results of these experiments were summarized in the article as follows:

1. In the hypophyses of 17 Native mine workers who died of primary carcinoma of the liver, intracytoplasmic inclusion-bodies were present. These bodies could not be demonstrated in Natives of the same tribes who died at the same time of other diseases.

2. In the sub-inoculated guinea-pigs and rabbits these inclusion-bodies appeared in the cytoplasm as well as in the nuclei of the host cells in various organs.

3. The nature of these inclusion bodies is not fully understood, but it is regarded as significant that they appeared in the patients as well as in the experimental animals.

4. From these preliminary observations the question arises whether these inclusion-bodies represent inclusion-bodies of a virus. More work is essential in order to establish their true nature, and to come to a conclusion whether these organisms are capable of contributing in any way to the formation of malignant tumours.

INCLUSION BODIES

In June 1951 I was able to resume my research work, which had been interrupted by the war. By kind advice of Dr. A. J. Orenstein, Dr. H. C. Berman provided me with the hypophysis of a Native mine-worker who had died of primary carcinoma of the liver with metastases in several other organs. Dr. T. Gillman—then of the Medical School Johannesburg—inoculated several guinea-pigs for me intraperitoneally with an emulsion of this hypophysis. These animals were killed 2 weeks later. In smears of their adrenals the same intracellular and extracellular bodies as described in the above article¹ were present in great numbers (Fig. 1).

I used an emulsion of the adrenals of one of these guinea-pigs for the inoculation of 7 other guinea-pigs.

From these guinea-pigs a great number of passages were obtained. Again in smears of the adrenals of all the inoculated animals which were killed or died spontaneously during my experiments the same bodies could be demonstrated. The smears were stained with Azur II-Eosin, Giemsa, Victoria Blue, or Gram's method (they are Gram-negative). Up to now (31 March 1956) I have seen the bodies in the adrenals of 162 guinea-pigs, and occasionally in the ovaries and in the liver. Reference is made in the present paper to experiments on guinea-pigs only, as the tests on other animals are not yet advanced far enough.

It is remarkable that the same bodies were encountered in the adrenals of guinea-pigs into which material

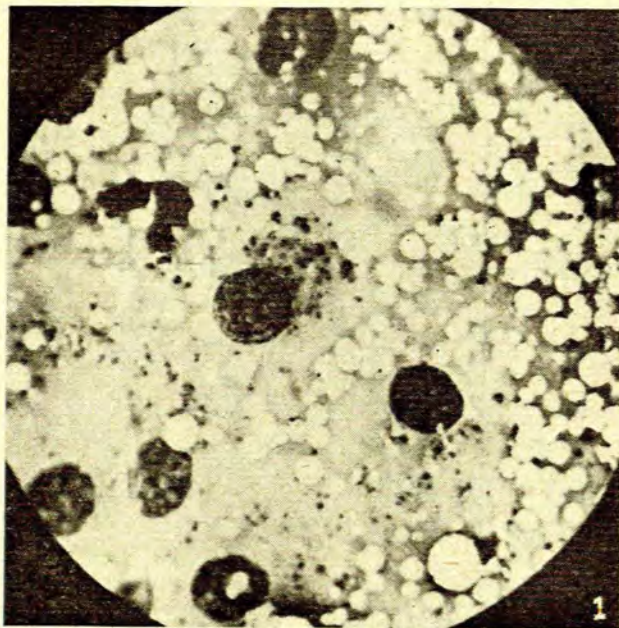


Fig. 1. Inclusion-bodies in smear of adrenals of inoculated guinea-pig (photomicrograph of this smear was published in Fig. 1 of Fischer, 1950¹).

from a dog was inoculated which died of carcinoma of the liver with multiple metastases in other organs. They, too, were transmitted from guinea-pig to guinea-pig—altogether in 25 animals. In the previous publication¹ it was stated, that the bodies were present chiefly in the medulla of the adrenals. At that time I had examined the adrenals only in smears. But numerous histological examinations have now clearly shown that their presence is confined to the zona reticularis of the cortex.

Histopathological examinations were carried out on organs of 36 guinea-pigs inoculated with the human strain and 7 with the dog strain. The specimens were cut and stained by Mr. Gerneke at Onderstepoort. The stains used were periodic acid Schiff (PAS), haematoxylin, Giemsa, Mallory and Gram. PAS proved to be the most useful stain, with which the inclusion bodies show a bright red colour (Fig. 2). They are

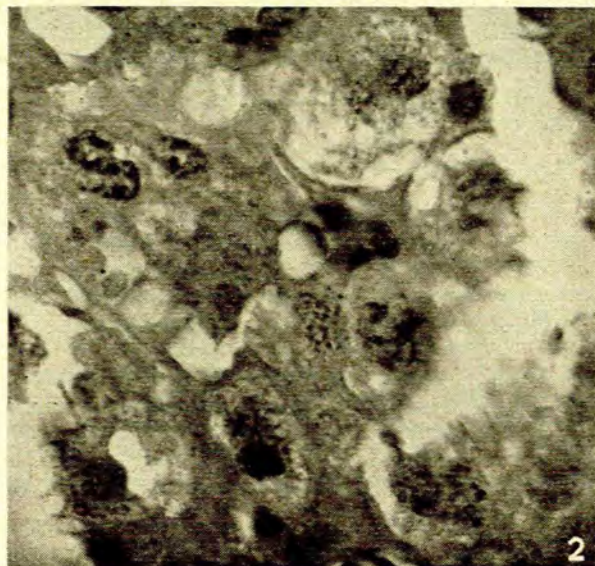


Fig. 2. Inclusion-bodies in zona reticularis of adrenal of guinea-pig inoculated with material of hypophysis of Native who died of primary carcinoma of liver.

more or less of the same appearance as described in my previous paper.¹ This was confirmed by Professor C. Jackson of Onderstepoort, who had the kindness to examine some of my specimens. He gave the following description of his findings in the adrenals of a guinea-pig the liver of which showed an early adenocarcinoma: 'Strictly confined to the deep zone (reticularis) of the cortex the epithelial cells show a spectacular content of cytoplasmic inclusion-bodies. When well developed they are often half the size of the nucleus. They are Gram-negative, react very doubtfully with Giemsa, and are strongly PAS positive. Indeed PAS is the only stain which shows them up. They look much like Negri-bodies (but the latter are not well stained with PAS). The larger ones are morulate, the smaller ones ring-like, with all apparent transitions to dust-like granules (? elementary bodies). Their relationship to material phagocytosed by the reticulo-endothelial cells

of the sinusoids would need closer study, especially whether the inclusion-body material is transformed into a pigment'.

The adrenals were usually enlarged and of a somewhat firm consistence, weighing from 200 to 500 mg., whereas the weight of normal guinea-pig adrenals ranged from 90 to 150 mg. (Fig. 3). In the adrenals

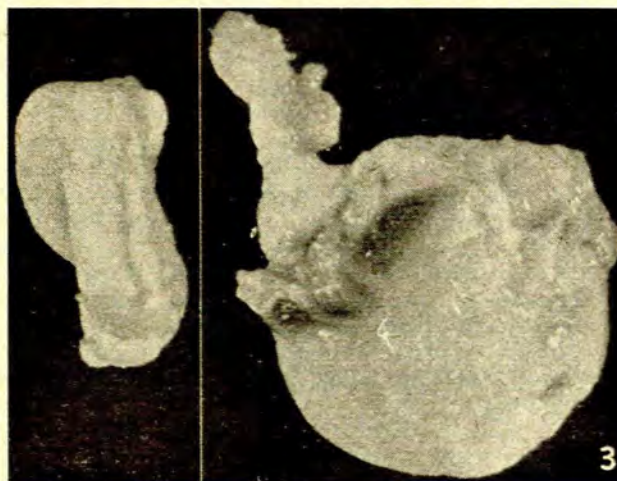


Fig. 3. Adrenals of guinea-pig. Right: heavily infected. Left: normal.

showing inclusion-bodies fatty degeneration and focal necrosis in the cortex were present.

CARCINOMA IN INOCULATED ANIMALS

In some of the inoculated animals very fine white nodules or tiny white spots were visible in the liver. In some others the surface of the organ was rough. In two animals coagulated blood was found in the abdominal cavity; the source of the bleeding could

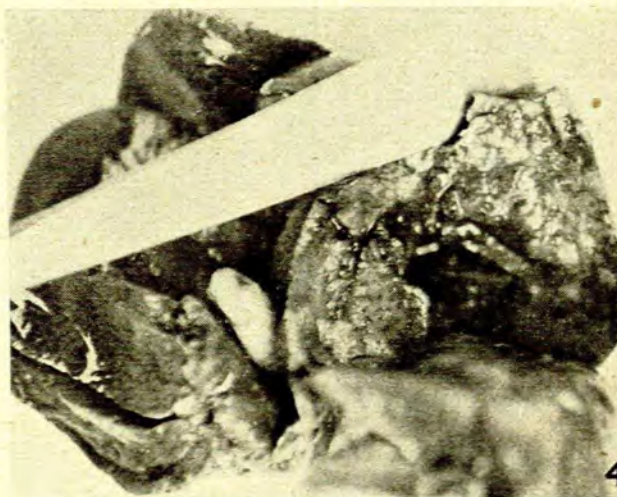


Fig. 4. Guinea-pig. Carcinoma of liver. Killed 52 days after inoculation. 2nd passage, human strain.

not be detected. One adenocarcinoma occurred in a guinea-pig of the 5th passage killed 30 days after inoculation. In this case the surface of the right lobe of the liver was covered with layers of fibrin. In the same lobe 2 small irregularly-shaped yellow-white patches and 2 very fine white nodules were present. The gross pathological changes in another case of carcinoma of the liver are shown in Fig. 4. This guinea-pig, a third passage, was killed 52 days after inoculation. Histological examinations of the organ revealed lesions ranging from congestion to pronounced fatty degeneration and multiple focal necrobiosis and necrosis, with more or less pronounced proliferation of bile duct. In several cases the normal architecture of the liver tissue was greatly disturbed and hardly recognizable. Very severe fatty degeneration combined with acute catarrhal intra-hepatic cholangitis was the cause of death of 3 guinea-pigs which died spontaneously during the experiments. Figs. 5, 6 and 7 show photomicrographs of carcinoma of the liver occurring in guinea-pigs inoculated with the human strain. An adenoma of the bile duct was found in the liver of one guinea-pig which was killed 3 years and 80 days after inoculation (see Fig. 8). In the spleen of this animal multiple foci of necrobiosis with intensive leucocytic infiltration, hyperplasia of the lymphoid tissue, proliferation of the sinus epithelium and congestion occurred.

The pathological changes in the organs of the experimental animals as described above are suggestive of the presence of a toxic substance produced by an infectious agent, which in my opinion belongs to the virus group. It is certainly not a type of rickettsia.

Not all the characteristics of a virus, especially those concerning filterability and cultivation, have yet been established satisfactorily, owing to technical difficulties. But there are properties of the infectious agent worked with which are similar to those of a virus. Its visible manifestations are cytoplasmic inclusion-bodies and very fine granules much like elementary bodies as seen

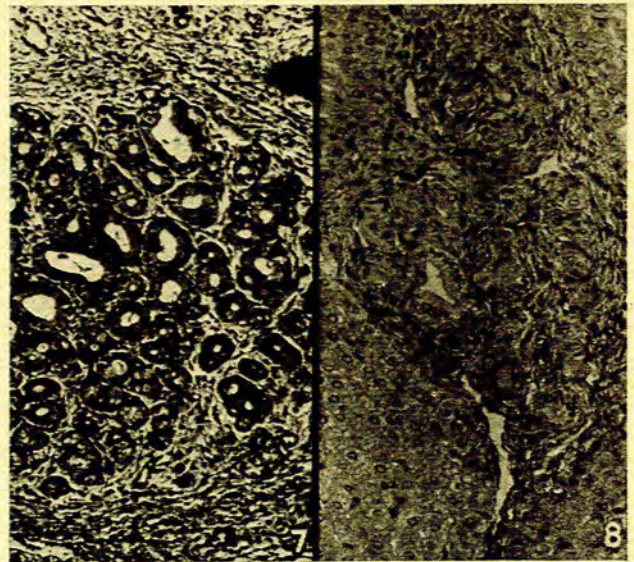


Fig. 7. Guinea-pig. Killed 27 days after inoculation. Human strain. Early adenocarcinoma of liver.

Fig. 8. Guinea-pig. Killed 3 years and 80 days after inoculation. 2nd passage, human strain. Adenoma of bile-ducts.

in other virus diseases. They can be found regularly in the zona reticularis of the adrenals of sub-inoculated guinea-pigs in any number of passages of the human strain as well as of the dog strain. Carcinoma of the liver, the disease from which the patients and the dog died from whom I obtained the material for my transmission experiments, was produced in several guinea-pigs. The virus apparently has an affinity for the glands of the endocrinal system. From a series of experiments, which are not yet completed, it seems possible to transmit the virus to certain plants.

PROTECTIVE ACTION OF SERUM FROM CANCER PATIENTS

Can this suspected virus cause cancer of the liver only, or has it any relationship with other types of cancer too? To investigate this question the following experiments were carried out: Guinea-pigs were inoculated simultaneously with sera of patients suffering from various types of cancer, together with emulsions of adrenals of guinea-pigs which contained numerous inclusion-bodies. Controls were inoculated with the adrenal emulsions alone. In 30 such experiments, carried out with the *human strain*, the animals were killed 4 weeks after inoculation. Of these experiments 6 came to a premature end owing to the death of one or the other of the animals. In 15 (62.5%) of the remaining 24 experiments inclusion-bodies did not appear in the adrenals of the animals inoculated in the manner described, whilst they were always present in the controls. The sera were obtained from 6 cases of carcinoma of the cervix, 2 of the breast, 2 of the head of the pancreas, and one each of the oesophagus, the stomach, the rectum, the colon, and the parotid gland. In the other 9 experiments (37.5%), in which the sera were obtained from 5 cases of carcinoma of the cervix, 2 of the stomach and one of the hepatic

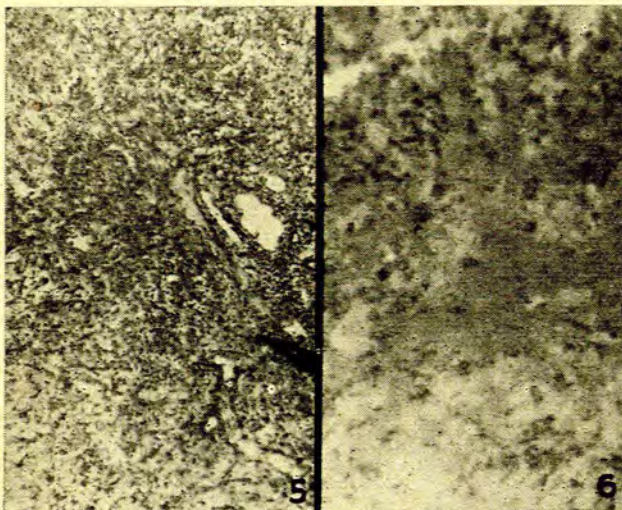


Fig. 5. Same guinea-pig as in Fig. 4. Carcinoma of liver.

Fig. 6. Same guinea-pig as in Figs. 4 and 5. Carcinoma of liver.

duct, both the inoculated animals as well as the controls showed inclusion-bodies in the adrenals.

In 3 similar experiments, with the dog strain the sera from one case of carcinoma of the cervix, one of the breast and one of the parotid gland were combined with strongly positive adrenal emulsions of the dog strain. Inclusion-bodies could be detected in the adrenals of the controls only. These sera had also been used with the same result in some of the experiments with the human strain.

All the sera in these experiments were provided from the clinical wards of Professors L. J. te Groen and J. K. Bremer of the University of Pretoria, to whom I am greatly indebted.

In 15 of the experiments with the human strain and in the 3 with the dog strain the sera of the cancer patients had apparently lowered or neutralized the virulence of the adrenal emulsions. In 9 experiments the sera had no inhibitory effect at all. From the results of the majority of these experiments it appears that the sera of patients suffering from various types of carcinoma may contain antibodies which can adversely affect the virulence of the suspected virus.

The experiments are of a somewhat empiric character. The adrenal emulsions were taken from different guinea-pigs, because to obtain such an emulsion the animal had to be killed. A few sera only were obtainable on any one day. Therefore no more than 2-4 sera could be combined with the same emulsion. Furthermore the content of antibodies in the sera must have varied considerably, as it depends on the stage and duration of the disease. In old-standing cases a weak content only or none at all can be expected. Accurate data in this regard were not always obtainable.

These experiments, which were of a preliminary nature, gave rise to more extensive research in the serological field, which is still proceeding. More work is essential in order to arrive at definite conclusions.

CONCLUSION

The interpretation of cancer as a virus disease would imply that it is not a disease affecting certain organs only, but an infection of the whole body. The virus may enter the body in similar ways to the germs of

other virus diseases. It may be harboured by healthy individuals, remaining harmless as long as the resistance of the tissues is not lowered—for instance by other diseases, malnutrition or old age. The early manifestations of the infection may be so slight and insignificant that they are easily overlooked or confounded with other conditions, inasmuch as in this stage full recovery may take place. Otherwise malignant growths may occur as a late symptom after the virus has invaded the hypophysis and other glands of the endocrinal system, upsetting their normal function.

SUMMARY

1. A description is given of the results of numerous transmission experiments carried out with material obtained from the hypophysis of 18 Bantu mine workers who died from primary carcinoma of the liver, and from a dog which died from carcinoma of several organs.
2. These observations suggest that this material contained an infectious agent of the virus group.
3. Visible manifestations of this suspected virus—inclusion-bodies—could be demonstrated in the adrenals of sub-inoculated guinea-pigs—human strain and dog strain alike—in many passages.
4. In several of these guinea-pigs true carcinoma of the liver was produced. In some others pathological changes were observed in the liver and in the adrenals suggestive of beginning malignancy. The lesions found in the organs of all the inoculated guinea-pigs are attributed to the presence of a toxic substance.
5. It appears that a relationship exists between this suspected virus and at least some of the other types of carcinoma.

I wish to express my gratitude to all the gentlemen already mentioned. Thanks are also due to Prof. J. Barnetson, Director of the Institute of Pathology of the University of Pretoria, for the many facilities placed at the disposal of the Cancer Research Laboratory of the Hans Merensky Trust. I am greatly obliged to Dr. W. K. Dannheimer for the numerous photomicrographs. Copies and enlargements of them, and the photograph of the gross specimen of the liver, were made by Mr. G. O. Kirsten.

REFERENCE

1. Fischer, W. O. (1950): S. Afr. Med. J., 24, 869.