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DOES THE BANTU LIVER CONTAIN CARCINOGENIC SUBSTANCES ?

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In 1940 Des Ligneris, of the South African Institute for Medical Research, published very interesting results concerning the problem of primary liver-cancer in the Bantu (Des Ligneris 1940). Unacquainted then with the work of the Shabad group in Leningrad (Shabad 1937, Kleinenberg, Neufach and Shabad 1940) on the subject of endogenic blastogenic substances, he considered it important to investigate the possible carcinogenic properties of liver extracts.

After having consulted Prof. E. L. Kennaway and Dr. I. Hieger (London) he prepared liver extracts from Bantu subjects who had died of primary liver cancer as well as from others without any symptoms of liver disease. An extract was also made of European livers.

TABLE I. RESULTS OF DES LIGNERIS' EXPERIMENTS

Extract	*No. of mice at beginning of experiment*	*No. of mice at end of experiment*	*Tumours*
1st series:			
Bantu with liver cancer	100	21	in 8 mice, 2 malignant.
2nd series:			
European, no cancer	100	72	0.
Bantu, no cancer ..	100	73	5 tumours, 2 malignant.
Bantu with liver cancer	100	71	12 tumours in 7 mice; 2 malignant.

These extracts were diluted with petroleum ether to 0·5% solutions and applied twice a week during 8 months to the skin of mice. At the end of the treatment skin tumours were seen in some of the mice painted with Bantu liver extract; some of them were considered to be malignant. In the groups of animals treated with European liver-extract or with cholesterol in petroleum ether (controls) no tumours appeared. Irradiation did not prove to have any influence so far as tumour formation was concerned. Des Ligneris' results are summarized in Table I.

Hieger (1940) injected some extracts sent from South Africa into mice and obtained a few tumours, but he did not publish any control experiments.

Steiner (1943) made an investigation with an extensive amount of material. He tested extracts of 67 different livers on 896 mice. Of these livers 37 belonged to patients with a malignant tumour, 13 to American negroes and 8 to subjects with cirrhosis of the liver. Unfortunately again a different method of preparing and applying the extracts was used: the total dose was given in 1-4 injections, mostly dissolved in sesame oil. Nothing suggestive of a difference in tumorigenic properties between the livers of Black and White was observed, nor were any tumours produced with extracts of cirrhotic livers, although as a rule primary liver cancer occurs in a cirrhotic liver. It must be remembered, however, that in the USA hepatoma is seen among negroes hardly more frequently than among people of European origin (Kennaway 1944).

AUTHOR'S EXPERIMENTS

It appeared useful to us to repeat Des Ligneris' experiments. In preparing the liver extracts his prescriptions were followed carefully: 'Add to every 100 grams of fresh liver 25 grams of solid NaOH together with 200 ml. distilled alcohol (free from admixtures). Heat this 15 minutes on a water bath, add an equal volume of distilled water and extract each 200 ml. of the mixture with 100 ml. of petroleum ether, which is distilled off. The residue is collected.'

Four different extracts were made, each of which was prepared from 400 g. of liver:

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Extract 1 was from the liver of a Bantu woman about 40 years old, who had died of pneumococcal meningitis. On microscopical examination the liver showed hyperaemia and some periportal infiltration. In the centre of the lobules quite a few vacuoles of fat were seen in the liver cells.

Extract 2 was from the liver of a 73-year-old White woman, who had suffered from severe adiposity and had died of embolism following pneumonia. The liver was enormously enlarged (2,700 g.) and under the microscope showed a pattern of acute congestion. In the liver cells quite a few vacuoles of fat were seen and there was a small increase in fibrous tissue.

Extract 3 was from the liver of a Bantu woman about 60 years old, who had suffered from cirrhosis of the liver with marked ascites and general emaciation. Microscopically this liver showed hyperaemia, an unmistakable increase in fibrous tissue, and a great many vacuoles of fat in the liver cells.

Extract 4 was from the liver of a 67-year-old White woman, in whom the pathologist had found a myocardial and a cerebral infarction, and bronchopneumonia and cirrhosis of the liver with haemorrhages. Microscopically a picture of Laënnec's cirrhosis was seen, with moderate morbid growth of gall-ducts and quite a few vacuoles of fat in the liver cells.

The extracts were at the beginning of the experiment diluted with petroleum ether (boiling range 100-120° C) till a 0·5% solution had been obtained. Four groups of mice were treated with the different extracts; a 5th group was painted with the solvent alone.

Whereas Des Ligneris made his experiments with common laboratory mice we were able to use mice of known qualities. They were called BCBA mice, the F_1 of the pure strains C 57 black (female) and CBA (male). At the beginning of the treatment the animals were not more than 4 months old—those of the 1st and 2nd group not more than 2 months old. They all received water *ad libitum* and well-balanced food, which contained different grains, vegetable fats, animal proteins, mineral salts and vitamins A, B and D.

Twice a week the mice were treated with the diluted extracts. The treatment consisted of twice painting a skin spot at the caudal end of the back, which had to be kept hairless with scissors. We could not confirm Des Ligneris' statement that the spot would remain naked through the influence of the petroleum ether.

Although we continued the painting of our mice during a time not shorter than Des Ligneris did, we did not obtain any tumours. It is true the epidermis of nearly all mice underwent changes in the painted area; first the skin grew red, then cornification began, and the cornified layer was shed in pieces, exposing a new and tender epidermal surface to the action of the liver extracts.

The regeneration of the skin did not proceed quite regularly in all the animals, but eventually in all surviving ones complete healing was observed. The skins of 3 mice which succumbed in this phase were examined histologically; no signs of abnormal epithelial growth were seen, but only decay of epidermis and cutis with some inflammatory reaction. The desquamative changes were seen after 4-5 months' treatment, except in the 4th group, where these changes were observed after only 2 weeks. In 2 of the mice of the control group the same skin-reactions appeared.

The general condition of our mice remained reasonably good. Only in the phase of desquamation did some animals die; so that our mortality rates, compared with those of Des Ligneris, are decidedly favourable. Only the hair grew thinner, for unknown reasons. Near the end of the treatment vaginal smears were made during a cycle, but no significant differences compared with the control group appeared to exist. Neither were any differences in body-weight found. In Table II certain figures are given reflecting the results of our experiments.

TABLE II. RESULTS OF AUTHOR'S EXPERIMENTS

Group	*No. of mice at the beginning of the experiment*			*No. of mice at end of experiment*	*Duration of treatment*
	Total	*Male*	*Female*		
Group 1 (Bantu) ..	46	28	18	43	40
Group 2 (European) ..	46	28	18	40	40
Group 3 (Bantu, cirrhosis)	36	21	15	34	33
Group 4, (European, cirrhosis)	37	19	18	37	33
Group 5 (controls) ..	20	10	10	18	30
	185			172	

We have no explanation for the results, which differ so much from Des Ligneris'. Our extracts were sufficiently fresh; painting began within 3 months after preparation of the extracts, which is not too long a time for substances made to be applied during 8 months at least. For technical reasons we painted the caudal part of the back, while Des Ligneris chose the area between the shoulder blades; this does not seem to be of great importance. The strain of treated mice is a more important factor, since strain differences in cancer susceptibility are well known.

SUMMARY

Des Ligneris' oncological experiments have been repeated. 165 BCBA mice were treated during at least 8 months with liver extracts from Bantu and European women who had died of liver cirrhosis or other diseases. No skin tumours were produced with these extracts.

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REFERENCES

Des Ligneris, M. J. A. (1940): Amer. J. Cancer, **39**, 489.
Hieger, I (1940): *Ibid.*, **39**, 496.
Kennaway, E. L. (1944): Cancer Res., **4**, 571.
Kleinenberg, H. E., Neufach, S. A. and Shabad, L. M. (1940): Amer. J. Cancer, **39**, 463.
Shabad, L. M. (1937): C.R. Soc. Biol., **124**, 213.
Steiner, P. E. (1943): Cancer Res., **3**, 385.