Pathology. — *On the isolation of a substance with carcinolytic properties from the reticulo-endothelial system* [1]). By N. WATERMAN and L. DE KROMME. (Communicated by Prof. G. VAN RIJNBERK.)

(Communicated at the meeting of March 26, 1927).

In an earlier memoir we demonstrated what FREUND and KAMINER had previously pointed out, namely that the normal blood-serum and the normal skin contain a substance capable of dissolving cancer-cells in vitro.

This property diminishes, or is totally lost, when a carcinomatous process arises.

Experimental research has opened up the possibility of studying the changes in this property on the dorsal skin of the tarred white mouse.

It has now been found the smearing with tar actually diminishes this lytic propertiy of the dorsal skin of the mouse, and that this property disappears altogether on the appearance of the carcinoma.

Further, the influence of Röntgen-rays upon this property appeared to be of great importance. If, for instance, skin-extracts possessed of a certain lytic power are subjected to Röntgen-rays, this power may be increased or diminished, according to the *length of time of the irradiation* (180 K.V., 2 m.A., 25 cm. unfiltered).

Inversely, a non-lytic extract from a carcinomatous skin may be made lytic again by röntgenization, and the same oscillations in the lytic action will be dependent upon the duration of the irradiation.

That this lytic power must have something to do with the disappearance of tumor-cells under the influence of irradiation, is evident from the fact that no destruction of the cells by the rays takes place in an inorganic environment, but does, on the contrary, occur when they are found in an originally non-lytic carcinomatous serum or organ-extract. (Biochem. Zeitschr. Vol. **182,** p. 377, and Fortschr. a. d. Geb. der Röntgenstrahlen, Vol. **35,** part. 4).

Whereas our results so far are more or less in agreement with those of FREUND and KAMINER, we differ from them widely on some points.

In particular their view that the lytic substance must be considered to be a certain saturated dicarbonic acid, we were unable to substantiate.

Neither succinic nor suberic acid, both of which may be taken as representatives of the above type of acids (as indicated by FREUND and KAMINER), exhibited any lytic action in our experiments.

---

[1]) After researches carried out in the Anthonie van Leeuwenhoekhuis at Amsterdam.

We are of opinion that the researchers in Vienna have been led astray by by-products.

We therefore felt constrained to take in hand once more the isolation of the lytic substance, and to do so after our own method.

Our hypothesis was that in the organism there must be a system which governs the generation and the regeneration of all the cells of the body, and which must be specially active wherever strong regenerative processes are continually at work.

We, therefore, determined in which organs the lytic element is present in the greatest quantity, and endeavoured to find a method of preparation whereby that element may be isolated from the organs. The organs examined were the liver, spleen, pancreas, thymus, adrenals and lymphatic glands.

Previous researches by FREUND and KAMINER had demonstrated that the active matter must be soluble in ether, and this was confirmed by us. But the experience with modern hormone preparations does not appear to warrant the conclusion that the substance has a lipoïd character.

Now, while the ether extracts of almost all organs were found to possess a more or less lytic action, the extracts of the lymphatic glands, spleen and thymus possessed this in a much higher degree than that of the other organs. This was in accordance with the views of various researchers who have attributed the power of resistance to carcinoma in particular to the quality of the connective tissue and to the reticulo-endothelial and lymphocytic apparatus.

First of all, however, the method of extraction had to be improved.

An extraction of calves' spleens and lymphatic glands was made with 10 to 15 times the quantity of ether under return-flow cooling for 8 hours. The ether extract was then partially evaporated and the last remains of the ether driven off by the air-current. The watery residue was treated with an excess of aceton. This caused a white flocculent precipitate, which was washed several times with aceton and finally dried on the filter. This dried precipitate was then rubbed down with 96 % alcohol (or methyl-alcohol), whereby the greater part is dissolved. After being filtered, however, a whitish-grey substance is retained, which is separated from the last traces of alcohol in the filter at 37 %. This whitish-grey powder is finally shaken briskly with a phosphate-buffer in phys. NaCl solution (1 part buffer-solution after SÖRENSEN of P_H 7.7 with 5 parts phys. NaCl solution). Hereby the active matter is dissolved.

In this way an aceton-precipitate of several hundreds of milligrammes is obtained from 500 Gr. of organ. After washing with alcohol, only fractions of one milligramme pass into the watery solution. This preparation, however, must be considered as still very impure. As a rule, a clear solution of the active matter can be obtained by this method. The presence of any traces of lipoïds hinders the determination of the lytic power. The solution

contains no ferment (trypsine: Fuld-Gross, or lipase: tributyrine test according to the stalagmometric method) and is devoid of albumen.

In determining the lytic power, account should be taken of the P_H of the environment in which this determination takes place. It appeared that an acid reaction counteracts the lysis; an alkaline (P_H of blood serum), on the other hand, promotes it. We therefore carried out all our determinations with a P_H of 7.1.

By this method a substance can be obtained from the spleen and lymphatic glands of the calf, which is able to dissolve cancer cells. The lytic power thus far has been brought up to 81 %. Further purification, which is very necessary, will probably raise this percentage still higher.

The question now was to see whether this artifically prepared extract would also be affected by the X-rays in the same way as serum or skin extract. This proved to be the case.

EXAMPLE:

Extract of lymphatic glands	Lytic power in %
Non-radiated	81
After 1 hour's irradiation	61
„ 2 hours' „	46
„ 4 „ „	62

SUMMARY.

1. Normal blood-serum and normal organs contain a substance capable of dissolving cancer-cells.

This substance is influenced by Röntgen-rays.

Extracts of carcinomatous organs lack this power, but obtain it by irradiation.

2. We have succeeded in isolating this substance from the reticulo-endothelial system, but as yet only in an impure state.

The solutions obtained contain neither tryptic nor lipatic ferment, and are free from albumen.

The lytic power of these solutions changes under the influence of Röntgen-rays.