CLXVIII. THE GLUTATHIONE CONTENT OF CHICKEN SARCOMA: A REJOINDER

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THE recent paper on "Quantitative studies of the nitroprusside reaction in normal tissues and tumours," by Kennaway and Hieger, appearing in this *Journal* [1927], seems to call for a brief comment.

A year ago we published our observations on the same subject [Yaoi and Nakahara, 1926] and reported that the Rous chicken sarcoma contains but little glutathione. This fact seemed especially interesting because of the abundant occurrence of glutathione in transplantable mammalian tumours, such as rat sarcoma and rat and mouse carcinomas, which are known to be similar to the avian tumour as regards their rate of tissue respiration and glycolysis [Murphy and Hawkins, 1925]. Kennaway and Hieger alleged that our results are incorrect, on the basis of the supposition that we used "glutinous and largely non-cellular material" from the centre of the overgrown tumours, instead of actively growing sarcoma tissue. This allegation is totally unfounded.

Nobody who is at all familiar with experimental cancer research would think of using central necrotic material for any study of tumour tissue. In our experiments we took the actively growing peripheral layer of the tumour, taking care not to include any adherent muscular tissue. The practice is so universally adopted by all experienced workers that we did not feel constrained to mention it in our paper.

In view of the quoted allegation, it is important to compare our figures. We reported three determinations, namely: 12, 13 and 20 mg. glutathione per 100 g. of fresh sarcoma tissue, based on the starch method. Kennaway and Hieger's figures for the same method ran: 9.6, 14, 20, 24, 27 and 54 in the case of intramuscularly implanted tumours, and 18, 21, 21, 24, 29, 30, 41, 47, 51 and 52 in those subcutaneously implanted.

We would call attention to the very great variation of their figures. Even allowing for a considerable experimental error, we can hardly see how such a wide range, as from 9.6 to 54 mg. %, for example, can be obtained by using any uniform material. From the information given it appears that they used only very young tumour grafts (6-11 days old) for their test. It is probable that their higher figures are attributable to the admixture of the reactive

tissue elements which, as is well known, are associated with the young tumour grafts, often in a considerable amount.

We feel obliged to point out another objection. Kennaway and Hieger report two instances where the nitroprusside method gave nil in both cases and the starch method 20 and 21 mg. %, and yet their data include two other cases in which the nitroprusside method gave 12 and 13 mg. %, while the starch method gave only 14 and 18 mg. % respectively. Are we to understand that, with the starch method, materials that gave 0 % with nitroprusside will yield a higher value than other materials that gave 12–13 mg. % with nitroprusside? This is an obvious self-contradiction, which seems to us to require some sort of explanation. In the face of these facts Kennaway and Hieger's figures do not seem reliable.

It seems to us that the difference between the results of Kennaway and Hieger and our own is not only apparent in the case of the Rous sarcoma, but also in the case of normal tissues, especially muscle. Tunncliffe [1925], whose method we are following, states that "the figures obtained for skeletal muscle have been exceedingly constant," and mentions 33–35 mg. % for rat, and 40–44 mg. % for rabbit muscle. We reported for chicken muscle 44 and 56 mg. %. These are in contradiction to the extremely low figures quoted by Kennaway and Hieger, namely: 16, 18, 19, 21 and 26 mg. %. These figures are so abnormally low and variable for this type of tissue that they lend themselves to the suspicion that they might be due to insufficient extraction. We ourselves have experienced some difficulty in finely grinding up muscle fibres.

Kennaway and Hieger conclude that human as well as animal tumours are not distinguished by any deficiency of glutathione. We are in agreement with this view, and we previously stated that glutathione is present in abundance in the malignant tumours, other than the Rous sarcoma, and in the normal tissues that we examined. However, the chicken sarcoma is exceptional in that its glutathione content is peculiarly small, considering its very active metabolism.

It may not be amiss to add here that our recent experiments show that the active layer of the Rous sarcoma has at best a very feeble reducing action on methylene blue [Yaoi and Nakahara, 1927]. Under the experimental conditions in which skeletal muscle of the chicken completely reduces methylene blue in 16 hours, the sarcoma tissue may fail to produce any perceptible reduction for 72 hours. If the glutathione content of the chicken sarcoma were as great or greater than that of the muscle, as claimed by Kennaway and Hieger, one would scarcely expect this exceedingly feeble reducing action on methylene blue on the part of the chicken sarcoma.

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