CLXXIII. THE SELECTIVE ABSORPTION OF ASCORBIC ACID BY GUINEA-PIG TUMOUR TISSUE.

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Bierich & Rosenbohm [1933] were the first to show that the greater part of the material titratable by iodine in tumour extracts was not glutathione. They also showed that liver and brain tissue contained something other than glutathione which reduced iodine solutions; they called this substance "*X*". Boyland [1933] found that the amount of the *X* substance present in tissue was equivalent to the 2:6-dichloroindophenol-reducing power and so appeared to be ascorbic acid. A note, however, was added to the paper in proof saying that biological tests indicated the presence of some other reducing substance in Jensen rat sarcoma tissue. The results indicated that if the indophenol reduction were entirely due to ascorbic acid, actively growing tumour contained 0·4 mg. ascorbic acid per g. whilst the necrotic tumour tissue contained very little. These results have been confirmed by Edlbacher & Jung [1934], Watson & Mitolo [1934] and Woodward [1935]. Harris [1933], however, claimed as the result of biological tests, of which the full details have not been published, that only about one-third of the indophenol-reducing material of Jensen rat sarcoma was ascorbic acid.

Borghi & Deotto [1934] gave extracts of the Ehrlich mouse sarcoma to guinea-pigs on a scorbutic diet and the animals died with scurvy. Bierich & Rosenbohm [1934], however, found that aqueous extracts of JRS tumours had antiscorbutic activity, but their experiments were not sufficiently quantitative to prove conclusively that the total reducing power was due to ascorbic acid.

Watson [1936] has tested the antiscorbutic activity of dried tumour tissue. Unfortunately drying of the tissue destroyed about one-half of the reducing activity. In the case of JRS he found that the residual reducing value was equivalent to the antiscorbutic activity. Dried Dael and Biltris guinea-pig tumour, on the other hand, had practically no antiscorbutic power although it reduced 2:6-dichloroindophenol. The fresh guinea-pig tumour had only half as much ascorbic acid as the rat tumour and on drying about three-quarters of the reducing power was lost. Watson also found that the reducing power of a guinea-pig sarcoma fell to one-third its normal value if the animal were kept on a scorbutic diet, and in the case of one animal the reducing power increased to the normal value when the animal was given 50 mg. ascorbic acid per day for 4 days. These two sets of experiments thus give contrary indications as to the ascorbic acid content of the Dael and Biltris sarcoma.

Kellie & Zilva [1936] in the foregoing paper show that the indophenol-reducing material of the JRS can be almost entirely accounted for as ascorbic acid as determined by combined biological and spectrographic tests. Experiments described in this paper show that this is probably also the case in the Dael and Biltris tumour.

It is now well known that the tissues of guinea-pigs subsisting on a scorbutic diet for a few days become depleted of ascorbic acid [De Caro, 1934; Zilva, 1935, 1, 2]. These workers have further shown that the tissues can be rapidly replenished by intravenous injection of ascorbic acid. The injected ascorbic acid is selectively absorbed by those tissues which normally are able to reduce indophenol preferentially, viz. anterior pituitary, adrenal, intestine and liver. The experiments described below show that tumour tissue is also capable of storing and absorbing ascorbic acid selectively.

Table I. *The apparent ascorbic acid content of guinea-pig tissues under different conditions.*

	Live wt. g.	Indophenol-reducing substances expressed as mg. ascorbic acid per g. tissue: Dael and Biltris tumour	Liver	Small intestine	Muscle	Carcass
(1) On normal diet	460	A 0·12 B 0·14	0·27	0·31	0·040	0·045
(2) On normal diet	375	0·15	0·25	0·20	—	—
(3) On normal diet	605	A 0·16 B 0·19	0·23	0·27	0·035	0·063
(4) After 8 days' scorbutic diet	410	A 0·025 B 0·030	0·050	0·040	0·020	0·040
(5) After 9 days' scorbutic diet	465	0·023	0·030	0·035	—	0·042
(6) After 10 days' scorbutic diet	690	0·023	0·039	0·034	0·014	0·016
(7) After 10 days' scorbutic diet	340	A 0·017 B 0·010	0·028	0·038	—	—
(8) 50 mg. ascorbic acid injected after 7 days' scorbutic diet. Killed 21 hours after injection	385	C* 0·025 A 0·10	0·20	0·31	0·018	0·022
(9) 50 mg. ascorbic acid injected after 8 days' scorbutic diet. Killed 22 hours after injection	610	A 0·062 B 0·070	0·21	0·21	0·032	0·030
(10) 50 mg. ascorbic acid injected after 8 days' scorbutic diet. Killed 24 hours after injection	385	0·092	0·26	0·21	0·040	0·060
(11) 50 mg. ascorbic acid injected after 9 days' scorbutic diet. Killed 23 hours after injection.	395	0·088	0·19	0·21	—	—
(12) 50 mg. ascorbic acid injected after 10 days' scorbutic diet. Killed 24 hours after injection	590	C* 0·009 A 0·12 B 0·11	0·25	0·44	—	—
(13) 50 mg. ascorbic acid injected after 10 days' scorbutic diet. Killed 22 hours after injection	475	A 0·12 B 0·15	0·23	0·27	0·021	0·052
(14) 50 mg. ascorbic acid injected after 10 days' scorbutic diet. Killed 22 hours after injection	725	A 0·11 B 0·13	0·60	0·40	0·034	0·048
(15) 50 mg. ascorbic acid injected after 11 days' scorbutic diet. Killed 24 hours after injection	495	A 0·08 B 0·13	0·20	0·31	0·027	0·032
(16) 50 mg. ascorbic acid injected after 11 days' scorbutic diet. Killed 22 hours after injection	610	0·15	0·24	0·27	0·027	0·045
Average of animals on normal diet	—	0·15	0·25	0·26	0·038	0·054
Average of animals on scorbutic diet	—	0·018	0·035	0·035	0·017	0·033
Average of animals on scorbutic diet after injection of ascorbic acid	—	0·11	0·27	0·29	0·028	0·041
(Average dry weight of tissue as % wet weight	—	16·5	28·3	14·5	24·0	—)
Average ascorbic acid content on normal diet calculated on dry weight	—	0·091	0·88	1·65	0·16	—
Average ascorbic acid content on scorbutic diet calculated on dry weight	—	0·11	0·12	0·24	0·071	—
Average ascorbic acid content on scorbutic diet after injection of ascorbic acid calculated on dry weight	—	0·67	0·96	2·00	0·12	—

A, B, and C refer to different tumours in the same animal. * C was removed before injection of ascorbic acid.

Experimental.

Guinea-pigs were grafted with the Dael and Biltris sarcoma (for the strain of which we are indebted to Dr Watson of the Imperial Cancer Research Fund). One month after the tumour had been grafted the animals were placed on the scorbutic diet described by Bracewell *et al.* [1930] for 8–10 days. The animals were then injected in the jugular vein under generalized ether anaesthesia [cf. Zilva, 1935, 2] with 50 mg. ascorbic acid dissolved in 1 ml. H_2O and neutralized immediately before injection. For gifts of ascorbic acid thanks are due to Prof. Szent-Györgyi and the Hoffman La Roche Chemical Works, Ltd. The urine of the animals was collected and ascorbic acid determined as a check on the injection technique. On the day following the injection of ascorbic acid the guinea-pigs were killed and the ascorbic acid content determined by titration of trichloroacetic acid extracts of the tissues with 2:6-dichloroindophenol, standardized against pure ascorbic acid. Other animals were examined after feeding with normal diet including cabbage *ad lib.* and others were killed and examined after having been fed on a scorbutic diet. The dry weight of the guinea-pig tissues was determined and the average ascorbic acid content of the tissues is given as calculated on wet and on dry weight basis (Table I). The average indophenol-reducing value calculated as ascorbic acid of the tumour tissue under conditions of ascorbic acid deficiency is 0·018 mg. per g. which is increased to 0·11 (sixfold increase) on injection of ascorbic acid. This increase is similar to that occurring with liver, 0·035–0·27 (eightfold), and small intestine, 0·035–0·29 (ninefold), and much greater than the increases found with muscle (twofold) or carcass (between two- and three-fold). The results show that tumour tissue selectively absorbs ascorbic acid and thus resembles liver and intestine which have been shown to fix ascorbic acid [Zilva, 1935, 1].

The ascorbic acid content of the tumours of animals which have been injected with ascorbic acid is not quite as high as in the case of animals fed on a normal diet. This is possibly due to the relatively poor blood supply of tumour tissue as compared with liver and intestine.

The extent of the variation of reducing power of tumour extracts with scorbutic diet and ascorbic acid injection makes it seem probable that the indophenol-reducing material of the guinea-pig tumour, as in the case of the rat tumour [Kellie & Zilva, 1936], is mainly ascorbic acid.

When the ascorbic acid content is expressed as a percentage of the dry matter of the tissue instead of the total weight, the ascorbic acid content of the tumour is seen to be of the same order as that of liver but much less than that of the intestine.

Summary.

The indophenol-reducing activity of a guinea-pig sarcoma like that of other tissues is greatly reduced when the animal is kept on a scorbutic diet; injected ascorbic acid is then selectively absorbed by the tumour tissue and those tissues which normally contain ascorbic acid.

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