XXIX. THE EFFECT OF COPPER COMPOUNDS UPON THE GROWTH OF CARCINOMA IN THE RAT.

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(Received February 2nd, 1926.)

SUGIURA and BENEDICT [1922] tested the effect upon the Flexner-Jobling rat carcinoma of 32 salts of a large number of metals (Na, K, Cu, As, Mg, Zn, Hg, Ca, Sr, Ba, Mn, Fe, Co, Mo, W, U, Se, Te, Ge, Pb, Zr, Os) given by the mouth. Of these 22 metals, copper showed the most definite inhibitory action upon the growth of the tumour. The metal was given as a watery solution of "CuSO₄" of strength up to 0.5 % mixed with the food; "each day a mixture of 50 parts of the basal diet and an equivalent of the salt solution was prepared."

The experiments described below consisted in the administration of copper by the method of Sugiura and Benedict to rats bearing an inoculated adenocarcinoma (Rat tumour 9 of the Imperial Cancer Research Fund), obtained through the kindness of Dr J. A. Murray. The original tumour of this strain arose in the abdomen, but the exact site of origin could not be determined.

EXPERIMENTAL.

The copper compounds were administered by the mouth. 100 cc. of solution of copper salt were mixed with 100 g. biscuit meal, which is then of a distinct greenish-blue colour. The copper solutions used were as follows: (a) CuSO₄·5H₂O in 0.3 % solution. The rats did not appear to be in good health when given a stronger solution (0.4 %). (b) Copper glycine, 0.25 %. (c) The solution obtained by mixing solutions of NaOH and CuSO₄ in the presence of sufficient cane sugar to prevent precipitation of cupric hydroxide ((b) and (c) were equivalent in copper content to (a)). The control rats received the same food but without added Cu. The duration of the experiment was usually from 2 to 3 months, depending upon the rate of growth of the tumour. In some series copper was given for 1 or 2 weeks before the grafting of the tumour.

When the rats were killed the alimentary canal from pharynx to anus was dissected out and rejected to avoid any contamination by copper in the food, the controls being treated in the same way. The bodies were minced, the tumours simply cut up with scissors, the mince being placed in silica dishes, dried at 110° and ashed over a Meker burner with flame collar. Unburnt carbon was oxidised by evaporating the ash to dryness with 5 cc. HCl and

5 cc. HNO_3 ; the residues were then brought into solution (cloudy) with just sufficient HNO_3 (bodies) or dil. H_2SO_4 (tumours). The solution, made up to 30–60 cc. and heated to 50–70°, was electrolysed at 2–2.5 volts in a 100 cc. beaker, the solution being stirred by a motor-driven glass paddle. Each electrode was of stout Pt foil, flag-shaped, 1 cm^2 , joined to a Pt wire. A serviceable electrode surface was obtained by treating the sand-blasted electrode successively with the finest brown emery powder, then with fused KHSO_4 and either boiling H_2CrO_4 or preferably HNO_3 and distilled water, and finally flaming at red heat. The copper deposited was removed from the cathode by raising it quickly from the solution with the current unbroken, rinsing with distilled water, and plunging into 5 cc. of a hot mixture of equal volumes of concentrated HNO_3 and water; this must be done as rapidly as possible or re-solution of the copper will occur. Electrolysis was considered to be at an end when no visible change on the surface of the cleaned electrode was observed after 1 hour's further passage of the current. The solution in HNO_3 was evaporated, ignited, and the residue dissolved in 1 cc. dil. H_2SO_4 , made up to 10 cc., and spun (Solution A). The residual fluid in the beaker was poured into excess of hot ammonia solution, the precipitated calcium phosphate filtered off, the solution evaporated to dryness, the evaporation repeated with 1 cc. H_2SO_4 to decompose nitrates, the residue dissolved in 1 cc. dil. H_2SO_4 , made up to 10 cc., spun, and the clear liquid (Solution B) used for colorimetric estimation of the trace of Cu not removed by electrolysis; this amount was seldom greater than 0.02 mg.

The estimation was made colorimetrically by the potassium ethyl xanthate method. Amounts of standard CuSO_4 solution containing from 0.0025 to 0.0125 mg. Cu were placed in tubes (20 × 50 mm.) and made up to 5 cc. with addition of 7 drops of 0.2 % fresh potassium ethyl xanthate solution. Larger amounts of Cu cause turbidity, whilst even much weaker solutions, in the presence of electrolytes and especially of oxidising agents such as nitrates, quickly become cloudy. For this reason separate colour comparisons were made with Solutions A and B since the latter must contain the salts of the ash not precipitated by ammonia. Experiments showed that the maximum change in colour intensity was obtained within the range of concentration 0.005 to 0.009 mg. Cu in 5 cc.; accordingly amounts were taken from the solutions A and B which would give a concentration of Cu within this range. The best form of comparator was found to be a box backed with thin white paper; ground-glass is unsuitable. The match must be made immediately after adding the xanthate before turbidity appears. Results for one ash (Solution A + Solution B) obtained by two independent observers seldom differed by more than 10 %.

The accuracy of this method was tested as follows. The ash from the body of a rat which had received injections of Cu glycine was dissolved with HNO_3 and made up to 100 cc.; to 50 cc. of this was added CuSO_4 solution equivalent to 0.075 mg. Cu (*i.e.* about $\frac{1}{4}$ of the copper content of the rat). The Cu content

of the two portions was then estimated in the manner adopted throughout these experiments: the Cu was recovered quantitatively.

	Mg. Cu found
50 cc. ash solution + added Cu	0.230
50 cc. ash solution alone	0.155
Difference	0.075

Seven blank estimations upon the reagents used in an estimation gave the following results: 0.018, 0.017, 0.027, 0.016, 0.020, 0.008, 0.020 mg. Cu, mean 0.018. This amount was subtracted from the results throughout. All the figures given in Table I are calculated from the mean of readings made by two observers independently.

Table I.

		Mg. Cu in 100 g. dry tissue	
		Bodies ¹	Tumours ²
I. CONTROL RATS			
	Rat 1	0.41	1.9
	2	0.29	1.6
	3	0.67	1.3
	4	0.42	2.7
	5	0.93	2.7
	6	0.40	2.9
	7	1.6	1.8
	8	2.2	—
	9	3.1	—
	Mean	1.11	2.13
	Mean of Nos. 1, 2, 3, 4, 5, 6	0.52	—
II. COPPER-FED RATS			
(a) CuSO ₄ -fed	10	0.59	3.2
	11	0.58	1.8
	12	1.3	4.7
	13	1.8	4.4
	14	1.0	—
	15	0.32	—
	16	0.96	—
(b) Cu glycine-fed	17	0.61	3.2
	—	—	3.6
	—	—	4.5
(c) Cu cane-sugar-fed	—	—	2.9
	Mean	0.90	3.54

¹ I.e. body after removal of tumour and alimentary canal.

² Each figure in this column refers to an analysis of the pooled tumours of several rats.

DISCUSSION.

The figures in Table I show that the average amount of Cu in the body apart from the gut and tumour cannot be greatly increased by giving Cu with the food; the copper-fed rats contained a mean of 0.9 mg. Cu %, and the whole series of controls actually more, namely, 1.11 mg. %. However, the control series includes three rats (Nos. 8, 9, 10) which have a very much higher Cu content than any of the others; no reason for this could be found. If these three be excluded, the mean of the control series is 0.52 mg. Cu %. The figures indicate, then, that the amount of Cu in the body at any one moment is not raised

very much even by food which is coloured greenish-blue with Cu salt; the body must therefore be very well protected against absorption of the metal. Even if the lower figure (0.52 mg.) be taken as correct for the normal, the increase in the copper-fed (to an average of 0.9 mg.) is an increase of only 75 %.

The tumours show a distinct difference between the two series; the tumours of the controls contain on an average 2.13 mg. Cu %, and those of the copper-fed rats 70 % more than this, or 3.54 mg. The figures in each series of tumours show much less wide variations than do those for the bodies of the rats; only one tumour in the copper-fed series contains less copper (1.8 mg. %) than the maximum amount in the controls.

From the therapeutic point of view the important question is whether copper absorbed into the body reaches a higher concentration in the tumour than in the other tissues. The averages in Table I as they stand indicate that the dried tumour of the copper-fed rats contains four times as much copper as the other dried tissues (3.54 : 0.9), and if the lower figure (0.52 mg.) be taken for the normal rats, their tumours and other tissues show just the same ratio (2.13 : 0.52, or 4 : 1). The amounts given in Table I are reckoned on the dried tissue, and the question must be considered whether such results are best expressed as percentages of the dry or of the fresh weight. The fresh body of the rat minus the tumour and gut contains a much higher percentage of solids (about 32 %) than does the tumour (average about 12 %, see Table II): the presence of adipose tissue and bone in the bodies may account for a large part both of this difference in water content, and of the difference in copper content shown by the bodies and tumours (Table I). The figures given below (Table II) show that the percentages of water in a series of samples of tumour tissue show no constant relationship to the amounts of Cu.

Table II.

Tumour tissue	Copper-fed					Controls		Mean
	1	2	3	4	5	6	7	
Total solids in 100 g. of fresh tissue, g.	12.0	22.0	8.7	12.0	7.7	6.0	13.0	11.8
Cu in 100 g. dry tissue, mg.	3.2	3.2	2.9	2.7	4.5	2.9	2.7	—

One may conclude, then, that the results are best expressed as percentages of the dried tissues, as in Table I.

In some of the earlier series of experiments with copper sulphate and copper glycine the metal seemed to have a definite inhibitory effect upon the tumour; an example is given in Table III. The rats were killed as soon as the skin over the tumour appeared likely to break; as there was no other cause of death in this experiment the time of survival after inoculation gives a rough numerical measure of the growth of the tumour.

The table shows a very distinct difference between the two series, especially about the 38th day, when 10 of the controls and only 3 of the copper-fed rats had been killed. Unfortunately in several later experiments the results have been less satisfactory. Large numbers of rats are necessary in this work in

order to correct for natural variations in susceptibility to the tumour. The data given in Table III probably show the maximum inhibition of this particular tumour which can be brought about by increasing the copper in it in the ratio of 2.1 to 3.5 (Table I) or by 66 %.

Table III.

Days after inoculation with carcinoma	Number of rats alive	
	Copper glycine-fed	Controls
0	15	15
34	15	9
38	12	5
52	7	3
88	7	3

The amounts of copper which can be absorbed from the alimentary canal seem then to be insufficient to exert any constant and considerable inhibiting effect upon this carcinoma. The exposure to X-rays of tumours containing such an increased amount of Cu as can be obtained by feeding (3.5 mg. % of dried tissue) would be of interest, as the metal would no doubt give rise to secondary radiations.

SUMMARY.

1. An electrolytic method for the estimation of amounts of copper in tissues of the order of 0.03 to 1 mg. is described.
2. A rat bearing a carcinoma and receiving no addition of copper to the food shows from two to four times as much copper % in the tumour as in the rest of the body, reckoned on the dry weight. The difference is perhaps due in part to the less cellular tissues (fat, bone) in the body.
3. When copper compounds (copper sulphate, copper glycine and copper sugar compounds) are given to carcinoma-bearing rats with the food for periods of 2-3 months, (1) the average amount of copper in the body is not increased by more than 75 %; large individual variations make it difficult to obtain exact results on this point; a few normal rats contain more copper than the average amount found in copper-fed rats: (2) the average amount of copper in the tumour can be increased by about 66 %. The ratio between the amounts of copper in the tumour, and in the rest of the body, is therefore not altered distinctly by giving copper with the food.
4. In some series of rats the administration of copper with the food appears to check the growth of carcinoma, but this effect is not obtained constantly.

This work was done during the tenure of a grant from the British Empire Cancer Campaign. I wish to thank Dr Leitch, the Director of this Institute, for allowing me the use of its facilities. My thanks are due to Dr E. L. Kenna-way, who carried out all the experiments on living animals and to whom I am also indebted in many other ways.

REFERENCE.

Sugiura and Benedict (1922). *J. Cancer Res.* 7, 329.