THE UPTAKE AND BINDING OF COBALT-60 AND ITS EDTA CHELATE BY RAT SKIN

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UNLIKE the liver which is normally assigned a prominent place, the skin is not usually considered to be very important in intermediary metabolism. But, in the course of our investigations, when we attempted to trace the path of cobalt-60 administered intravenously along with ethylenediaminetetraacetic acid (EDTA) in albino rats, we observed that there is an appreciable accumulation of radioactivity in the skin within a few minutes, the skin being the most preferred tissue in the body and the activity in the liver being negligible. Subsequently, the radioactivity rapidly leaves the skin and is excreted mainly through urine. This rapid passage of the EDTA-cobalt-60 chelate through the skin before being excreted is particularly intriguing, since we have observed earlier that this chelate is rapidly and completely excreted[2] and that in blood it does not combine with any blood protein, but exists as such,[3] which led us to assume that it probably does not react with any body constituent, but is directly excreted as such from the blood stream. With zinc-65 and iron-59 also, we have observed a similar passage of the EDTA chelate through the skin (to be published), and this seems to be common for all EDTA chelates, irrespective of the metal. In view of these observations, we have studied the uptake of cobalt-60 by skin slices *in vitro* and also the nature of the binding of cobalt-60 and its EDTA chelate in the skin. The results are reported here.

Fresh skin from adult albino rats, after removal of hair and washing with saline, is cut into thin slices and used. 300 mg. lots of the slices are incubated for an hour with 0·5 ml. of cobalt-60 tracer (as cobaltous chloride solution, approximately 2000 c.p.s.) and 1 ml. of 0·1 M phosphate buffer of the desired pH the reaction being stopped by the addition of 1 ml. of 30% trichloroacetic acid. The radioactivity in the precipitate is determined after centrifugation, washing and digestion with nitric acid. The optimum pH is found to be 7·8 and the uptake for one hour at this pH is 17% of the activity in the incubation mixture.

The effect of EDTA on the uptake of cobalt-60 has been studied by the addition of varying amounts (0·1 to 0·5 ml.) of 10 mM EDTA solution to the incubation mixture. The total volume of the incubation mixture is kept constant at 2·0 ml. by the addition of suitable amounts of isotonic saline. At all concentrations tried, EDTA has been found to be strongly inhibitory, the uptake being less than 20% of that of the control. This inhibitory action of EDTA is in sharp contrast to its effect *in vivo*, where a preferential deposition of radioactivity in the skin occurs. EDTA also inhibits completely the uptake of cobalt-60 by liver slices[4] or lung homogenate.

The stability of the binding of cobalt-60 in the skin has been studied through dialysis. Dialysis for 24 hours results, on an average, in the removal of 26·7% of the radioactivity deposited *in vivo* in the skin, of 32% of the activity taken up *in vitro* by normal skin slices or of 27·6% of activity taken up *in vitro* by previously-boiled skin slices. This suggests that cobalt-60 exists in the skin mostly in the bound forms even as in the liver.[4] Also, the amounts removed through dialysis are nearly equal in all the three cases. This implies that the nature of binding is probably identical in all of them.

The binding of cobalt-60 in the skin, ten minutes after intravenous administration as the EDTA chelate, has also been studied. About 50% of the radioactivity in the skin is removed by dialysis for 48 hours, but the amount of activity removed decreases progressively with time so that during the next 48 hours, only about 10% is removed. As such, even at the end of prolonged dialysis for 96 hours, nearly 40% of the original activity still remains in the skin. This strongly suggests that the cobalt-EDTA chelate is at least partly bound to skin proteins. Thus the cobalt-60-EDTA chelate in the skin, with its incomplete dialysability, is in sharp contrast to a mixture of cobalt-60 and EDTA solutions or to the cobalt-60-EDTA chelate in blood, both of which have been observed to be freely dialysable, all the radioactivity being nearly completely removed within

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21 hours.[3] This would imply that, while the EDTA-cobalt chelate does not react with any blood proteins, it undergoes at least partial binding in the skin before it is excreted. Similar evidence for a partial binding of this chelate in the bones and the muscles has also been obtained.

The radioactivity dialysing out from the skin in the above instance can be either cobalt-60 in the ionic form or its EDTA chelate or both. In an attempt to identify the exact forms, the dialysate is concentrated to a very small volume and subjected to electrophoresis on paper strips for 150 minutes in a 'Shandon' vertical-type electrophoresis unit using a current of 25 milliamperes and veronal buffer pH 8·3. Under these conditions, while the ionic form stays very much near the point of application, the chelate moves over 1½" towards the anode affording a clean separation. After electrophoresis, the paper strips are dried, the various areas marked, cut into pieces and counted for radioactivity. All the activity dialysed out is found to be present completely as the EDTA chelate. This supports our view that cobalt-60, administered as certain chelates, moves from one tissue to another in the form in which it is administered—may be in combination with some body constituents, like blood proteins, but never splitting up into the ionic form.

We are grateful to the Director Dr. S. Krishnamurthi, M.S., for his keen interest in this work and to the Department of Atomic Energy, Government of India, for financing this research project.

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SOME OBSERVATIONS ON THE CYTOLOGY OF *NICANDRA PHYSALOIDES* GAERTN.

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DURING the course of a study of the morphology of pachytene chromosomes in some Solanaceæ observations were made on the cytology of the monotypic genus *Nicandra physaloides*, var. *immaculata*, raised from seeds obtained from the Royal Botanic Gardens, Kew, and some deviations from those made by Darlington and Janaki Ammal[1] have been met with which are recorded in this note.

Darlington and Janaki Ammal[1] confirmed the previous chromosome counts of $2n = 20$ made by Vilmorin and Simonet[4] and Janaki Ammal[2] and recorded the occurrence of a pair of isochromosomes with identical satellited arms. These were present in addition to the regular nucleolus organising chromosome pair included in the remaining nine pairs of 'autosomes' such that there were six nucleolus organisers in the complement of the species. The plant investigated by them was therefore interpreted as having "the unique property of being regularly tetrasomic for a part of its chromosomes and disomic for the rest" at the diploid level. They have also recorded (*i*) frequent inside pairing of the isochromosomes leading to univalent formation, (*ii*) irregular distribution of the univalents at anaphase I leading to the formation of microspores and megaspores deficient for the isochromosome and (*iii*) occurrence of progeny with $2n = 19$ chromosomes derived from the fertilisation of a deficient egg nucleus ($n = 9$) by a normal sperm ($n = 10$).

In the material studied by us it was found that at pachytene there is a single nucleolus organising bivalent attached to the nucleolus (Fig. 1, *a* and *b*) and this is the only satellited chromosome pair in the complement. There were no isochromosomes in any of the pollen mother cells examined. The chromosome counts, somatic as well as meiotic, however, revealed the presence of $2n = 20$ and $n = 10$ chromosomes uniformly. Careful examination of sixty nuclei at diakinesis showed a regular formation of ten bivalents in each of them (Fig. 2) and no