tope was preferentially excreted into the urine as compared to the other. The U/T ratio averaged 1.9 times higher for ^{131}I than for ^{125}I in the rats fed LID and 1.4 times higher in the rats fed the high-iodine diet. The lower value in the latter group is consistent with the well-known lower rate of extraction of iodide from the plasma by the thyroids of iodine-replete than of iodine-deficient animals (*8*). The kidney and the thyroid are the two principal competitors for iodide from plasma. Thus, a decrease in the rate of iodide uptake by the thyroid permits an increase in the quantity of iodide excreted in the urine. The higher the relative rate of urinary excretion of iodide from plasma compared to that of thyroid accumulation of iodide from plasma, the less difference there will be in the U/T ratios of iodide originating as plasma iodide or through renal deiodination of T_3.

These results support the validity of our hypothesis that a portion of the iodide derived from T_3 deiodination in the kidney can enter the renal tubule lumen and be excreted in the urine without being reabsorbed to mix with the body iodide pool. Presumably iodide derived from T_4 or iodotyrosine deiodination in this organ would be handled in a similar manner.

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Fluorescent Banding Patterns of Rat Chromosomes in Normal Cells and Primary Hepatocellular Carcinomas

Abstract. *Quinacrine staining permits identification of rat chromosomes in metaphase that were formerly classified only in groups within the karyotype. This technique defined two types of abnormal chromosomes in cells of rat hepatomas.*

The binding of quinacrine compounds to chromosomes has been described for several species of plants and animals (*1*). The fluorescent banding patterns of these chromosomes complexed with quinacrine appear to be constant for a given species. This complexing technique is therefore a useful addition to standard karyotype analysis for identification of individual chromosomes. Its applications include: (i) identification of individual chromosomes (*2, 3*); (ii) characterization of structurally or numerically abnormal chromosomes (*3*); and (iii) identification of the species of origin of chromosomes in hybrid cells (*4*).

The fluorescent banding pattern for rat chromosomes has not yet been described. We have examined this pattern to make specific identifications within groups of rat chromosomes that are otherwise morphologically indistinguishable. We present here a description of this pattern, which we have used to analyze the chromosomes in cells of primary hepatocellular carcinomas (hepatomas) that were induced in rats by chemical carcinogens.

All cells examined came from several lines of Sprague-Dawley rats. Lymphocytes from males and females were examined after 4 days of culture in vitro with phytohemagglutinin. The hepatomas were induced in the rats by the ingestion of *N*-2-fluorenylacetamide (*5*). Cells in metaphase were obtained directly from the hepatomas, and the preparation of these cells and lymphocytes has been described (*6*).

Dividing fibroblasts (*7*) were examined at weekly intervals during 4 months of cultivation in vitro. These rat embryo cells, originally prepared from a single, minced, 18-day embryo, were maintained in cell culture in Dulbecco's 4X medium with 10 percent calf serum. Cul-

Fig. 1. A graphic representation of the chromosomes of the rat; haploid set +X+Y. The autosomes are numbered. The light bands within the chromosomal outlines represent the fluorescent bands produced by quinacrine staining. This diagram is based on a study of 61 cells with relatively extended chromosomes; some of the bands appear fused in contracted chromosomes.

tures were divided and transferred twice weekly.

Chromosome preparations (Fig. 2A) were stained with either quinacrine (Q), obtained as quinacrine dihydrochloride (Atebrin-Gurr), 5 mg per milliliter of water, or with quinacrine mustard dihydrochloride (QM) in water (*8*) (50 μg/ml), after prior treatment in McIlvaine's buffer, *p*H 5.6 (Q), or *p*H 4.1 (QM); the cells were then mounted in buffer according to the methods of Caspersson (*1*). Light from an HBO 200 mercury lamp was passed through a BG 12 or FITC exciter filter and transmitted through the specimens. The light was then filtered by a K 530 barrier and the preparation was examined with the use of a Zeiss Photoscope with a vertically mounted camera.

The normal, orcein-stained, metaphase spread from a rat diploid cell permits limited chromosome identification. The three largest chromosome pairs are identifiable individually on the basis of size and morphology. The next nine pairs are acrocentric chromosomes in order of decreasing size. Both X and Y chromosomes fall within the morphologic limits of this group. Chromosomes 13 to 18 consist of six pairs of metacentric chromosomes of which the two largest are submetacentric. Chromosomes 21 and 22 are pairs of small metacentric chromosomes. Satellites are seen, and help to identify, pair Nos. 3 and 12. Thus, where standard techniques are used, positive identification of pair Nos. 1, 2, 3, and 12, and occasionally 13 and 14, is possible (Fig. 1).

Diploid cells from the sources described, including the hepatomas, demonstrated a consistent fluorescent banding pattern that allowed positive identification of many of the previously indistinguishable pairs of chromosomes. The distinguishable pairs (Nos. 1 to 3) indicated, by the consistency of their fluorescent banding patterns (Figs. 1 and 2), that the banding was uniform in the various cell types examined. Precise identification of pair Nos. 4 to 7 and No. 11 could be made on the basis of the fluorescent banding pattern. In pair Nos. 8 through 10 the banding pattern was frequently faint and difficult to distinguish. The sex chromosomes were distinguishable with this technique. The X chromosome, similar in size to chromosomes 3 to 7, was easily identifiable in a cell from a male as an unmatched chromosome with bright fluorescence over most of its long arm and an unstained region at the distal end of it. The Y chromosome, similar in size to the No. 12 pair in many cells, but without satellites, was diffusely stained by the fluorochromes. The intensity of fluorescence of the Y chromosome varied widely from cell to cell in a single preparation and only rarely was brighter than all other chromosomes [in contrast to published reports of other species (*9*)].

Fluorescent stains were less helpful in distinguishing among the small metacentric chromosomes. Distinctive bands identified chromosomes 13 and 14, and a large, very brightly stained region comprised most of one arm of the No. 15 pair. A similar, but less intense, fluorescence over one arm was seen in the No. 17 pair. The remaining small metacentric chromosomes stained poorly and could not be differentiated clearly in our material.

Among the quinacrine-stained chromosomes certain areas characteristically

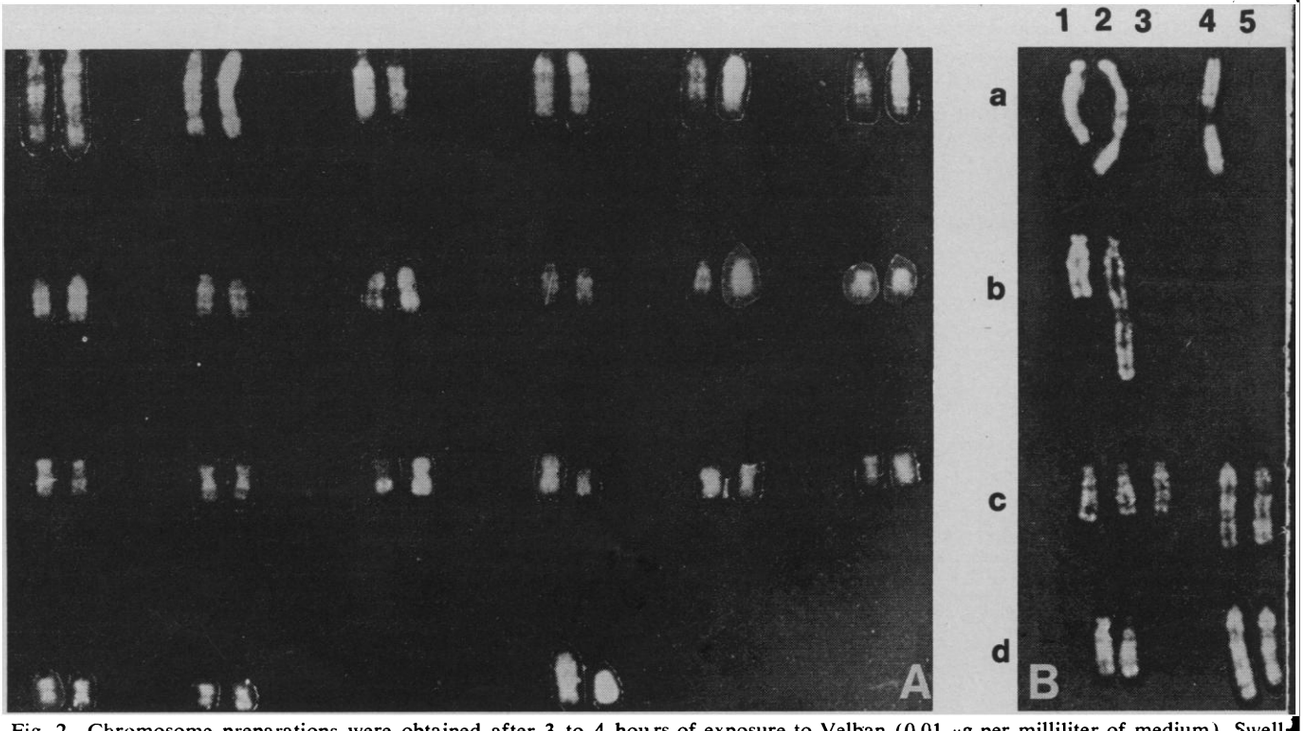

Fig. 2. Chromosome preparations were obtained after 3 to 4 hours of exposure to Velban (0.01 μg per milliliter of medium). Swelling was induced by exposure to calf serum diluted with distilled water (1:5). Recently, we have used 0.38 percent KCl, which improves alignment of chromatids. (A) Karyotype of a rat fibroblast, stained with quinacrine, demonstrating the fluorescent banding pattern typical of a diploid cell from a male rat. (B) Each row (*a–d*) shows chromosomes from a different hepatoma cell. The columns in this figure are labeled numerically for ease of description. The chromosome on the far left of each cell (at *a1* to *d1*) displays fluorescent banding typical of a normal No. 1 chromosome. The chromosome at *a2* is a large dicentric, apparently the result of fusion of a No. 1 with a No. 8 chromosome. The chromosome at *a4* is a large abnormal metacentric chromosome. A translocation in cell *b* resulted in the large anomalous No. 1 chromosome at *b2*. The last two cells contain, respectively, three (at *c1, c2, c3*) and two (at *d1, d2*) normal No. 1 chromosomes. Both of these cells also include pairs of large telocentric marker chromosomes (at *c4, c5* and at *d4, d5*) with repeated wide bands of bright fluorescence, a pattern not seen in normal rat chromosomes

give bright fluorescence. In the rat metaphase cell these areas included portions of the long arms of chromosomes 2, 3, and 7, a paracentric band on the long arm of chromosome 14, and one arm of chromosome 15. The Y chromosome appeared polymorphic in intensity of staining.

Interphase nuclei from species in which the Y chromosome stains intensely with fluorescent dyes have characteristic, bright fluorescent spots (*9*). We examined rat interphase nuclei from tissue cultures and peripheral blood cultures of male origin (approximately 1000 cells per culture). We found bright spots in 3 to 5 percent of such cells but most of the nuclei exhibited only a somewhat uniform granular staining. Occasionally, large, but poorly defined, masses within the nucleus stained with greater intensity.

Most of the hepatomas examined had a bimodal population of dividing cells, with varying proportions of diploid cells and the remainder clustered about a hypotetraploid mode (*6*). The fluorescent banding patterns in the diploid tumor cells were comparable to those of diploid cells from normal sources, and we could find no evidence of structural rearrangements.

Structural rearrangements were observed in the hyperploid cells and in some instances the chromosomes that contribute to large marker chromosomes could be identified. For example, a dicentric chromosome could be identified, by its banding pattern, as the fusion product of a No. 1 chromosome with a smaller acrocentric chromosome, probably a No. 8 (Fig. 2B). In other tumor cells, however, we observed abnormal chromosomes that did not exhibit the fluorescent banding patterns of normal chromosomes. For example, a pair of large telocentric markers were found in most metaphase spreads from one of the tumors. In one such cell with 86 chromosomes the four No. 1 chromosomes showed the expected banding patterns, but the marker chromosomes showed a repeating pattern of broad bands of fluorescence not seen in normal cells (Fig. 2B).

Much of the previous difficulty in identifying specific chromosomes in the rat karyotype has been overcome by use of the flourescence-banding technique.

Recognition of the normal fluorescent banding pattern of rat chromosomes can serve to identify translocation exchanges that would otherwise be undetected and to identify those chromosomes that contribute to the formation of new marker chromosomes in malignant cells. The former is of great interest for analysis of diploid and "pseudodiploid" tumors. The importance of the latter (identification of the origin of marker chromosomes) relates to the survival value of such chromosomes in that we have seen markers in many chemically induced hepatomas (*6*) and they have also been reported in Rous sarcomas (*10*).

By using a fluorescent staining technique, we have been able to describe the normal chromosome banding-pattern for the rat and to define two types of abnormal chromosomes in rat hepatomas. One of these abnormal types is clearly the result of fusion of normal chromosomes but the origin of the other is unexplained.

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6,7-Dihydroxytetrahydroisoquinoline: Uptake and Storage by Peripheral Sympathetic Nerve of the Rat

Abstract. *6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline is a pharmacologically active alkaloid that can be formed by condensation of dopamine with formaldehyde. We used fluorescence microscopy to study in vitro the uptake and storage of this compound by sympathetic nerves of the rat iris. Rats were treated with reserpine or with the methyl ester of α-methyl-p-tyrosine in order to deplete the endogenous catecholamine stores. Accumulation of the alkaloid was about one-tenth that of norepinephrine. Uptake was completely blocked by 10^{-5}M desmethylimipramine. These results offer some explanation for the sympathomimetric properties of the alkaloid. Similar results can be expected for similar tetrahydroisoquinolines that may be formed in vivo from endogenous catecholamines during ingestion of alcoholic beverages.*

1,2,3,4-Tetrahydroisoquinoline (TIQ) alkaloids are synthesized in isolated intact adrenal glands from cows as a result of a condensation reaction between endogenous catecholamines and perfused acetaldehyde (*1*). Similar TIQ products are formed from dopamine (DA) and acetaldehyde in rat tissue homogenates (*2*) or from endogenous catecholamines during perfusion of cow adrenal glands with formaldehyde (*1*). In vivo, TIQ's are synthesized in the adrenal medullas of rats during administration of methanol (*3*), which is a metabolic precursor of formaldehyde. It has been suggested that TIQ alkaloids can be biosynthesized in the adrenals and the adrenergic neurons of man during ingestion of alcoholic beverages and that these alkaloids may alter adrenergic function (*4*). We report that 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (6,7-dihydroxy-TIQ), the alkaloid formed by condensation of DA with formaldehyde, is taken up and accumulated in vitro by sympathetic nerves of the rat iris. The amount of alkaloid accumulated was less than that for norepinephrine (NE) but greater than that for DA. Uptake was blocked by low concentrations of desmethylimipramine (DMI). Thus, 6,7-dihydroxy-TIQ has properties akin to those of the catecholamine neurotransmitters and may be capable of altering activity of sympathetic nerves.

We used fluorescence microscopy to study the accumulation of DA, NE, or 6,7-dihydroxy-TIQ in the sympathetic nerves of the iris of the rat. In this well-known method (*5*), moist formaldehyde gas is used to transform the