

results in an increased ratio of oxide to vitamin K₁ and the oxide inhibits the activity of the vitamin by competing with it for an active site (Fig. 2). We have demonstrated that both the conversion of vitamin K₁ to the oxide and the reverse reaction occur extensively in normal rats. In animals treated with warfarin the conversion of oxide back to vitamin K₁ is blocked².

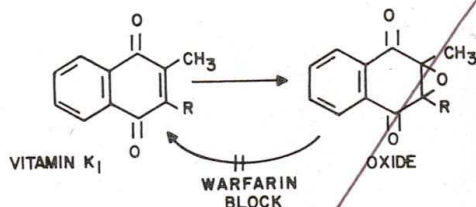


Fig. 2 Effect of warfarin on interconversion of vitamin K₁ and oxide. R, phytol side chain.

These results support the idea that vitamin K₁ and the oxide bind competitively at an active site. In contrast, there is clearly no simple competitive relationship between vitamin K₁ and coumarin anticoagulants⁸. Lowenthal *et al.*^{8,9} found that both *in vivo* and *in vitro* warfarin inhibits the stimulation of factor VII synthesis at low doses of the vitamin and anticoagulant. As the doses increase while the ratio is maintained, inhibition disappears. It was proposed that at high concentrations vitamin K₁ can enter cells by a route that is not sensitive to coumarin, but these results can also be explained by our model invoking the inhibitory effects of phyloquinone oxide. At low doses of vitamin K₁ and warfarin, a large part of the vitamin is converted to the oxide and the oxide/vitamin K₁ ratio is high. At high doses of vitamin and warfarin, a relatively smaller part of the vitamin is converted to oxide and the ratio is not high enough to cause inhibition. Preliminary evidence indicates that there are different enzymes located in different subcellular fractions catalysing the conversion of vitamin K₁ to the oxide and the reverse reaction¹⁰.

The inability of large doses of phyloquinone oxide to overcome the inhibition of prothrombin synthesis is somewhat surprising in view of its former clinical use as an antagonist of 'Dicumarol'¹¹⁻¹³. Miller *et al.*¹³ found that a commercial preparation of phyloquinone oxide (Merck and Co., Rahway, New Jersey) had little activity in dogs treated with 'Dicumarol', but large oral doses were apparently effective in patients on 'Dicumarol' therapy. The ability of the commercial preparation to overcome the inhibition of clotting factor synthesis by the anticoagulant was possibly due to contamination with vitamin K₁ since the oxide is prepared from vitamin K₁ and they are very difficult to separate¹. The phyloquinone oxide used in our studies was rigorously purified and identified¹.

The availability of humans and rats which have a hereditary resistance to warfarin has provided insight into the mechanism of action of the anticoagulant. The resistance is apparently not due to a change in the uptake, rate of excretion, or metabolism of the drug¹⁴⁻¹⁸. Hermodson *et al.*¹⁶ found that the resistant rat had twenty times the requirement for vitamin K₁ to maintain normal prothrombin levels. They postulated that the resistance results from a mutation in a receptor protein causing it to have a lowered affinity for both vitamin K₁ and warfarin. In support Thierry *et al.*¹⁷ found that ribosomes isolated from livers of normal rats contained three to five times as much labelled warfarin as those isolated from warfarin-resistant animals. The subcellular distribution of phyloquinone, however, was not significantly different in normal and resistant animals. From clinical studies on warfarin-resistant families, O'Reilly has also proposed a genetic mutation of the vitamin K-anticoagulant receptor site¹⁸. According to our proposed model for the action of warfarin, resistance in man and rats could be due to a mutation in the enzyme which converts the oxide to vitamin K₁ causing a reduced sensitivity to warfarin.

An effect of warfarin on the metabolism of vitamin K₁ does not rule out the possibility of a direct effect of the anti-coagulant on the stimulation of the synthesis of clotting proteins by the vitamin. Studies on the metabolism of vitamin K₁ and phyloquinone oxide in resistant rats should clarify this issue.

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ROBERT G. BELL

Biochemistry Department,
University of Rhode Island,
Kingston, Rhode Island

JOHN T. MATSCHINER

Biochemistry Department,
School of Medicine,
University of Nebraska,
Omaha, Nebraska

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Marker Band in One Chromosome 14 from Burkitt Lymphomas

KNOWLEDGE of the detailed pattern of fluorescence of the normal human karyotype, showing more than 200 bands per haploid chromosome set¹, has enabled us to recognize, both in biopsies and in cell cultures from several Burkitt lymphomas, an extra band in one homologue of D group chromosome pair No. 14. The deviation was seen in all analysable cells of five out of six tumour biopsies and of seven out of nine tumour cell lines examined, representing twelve different tumours from nine male and three female patients. In three tumours both biopsies and cultures were examined, and it was found that all gave results consistent in the two types of samples. Thus, two of them had the marker band in both the biopsy and culture, the third revealed the marker band absent in both cases. The remaining tumours were investigated only in biopsies or only in culture. They were positive in eight cases, negative in one. Of the twelve tumours examined altogether, ten were positive and two negative.

The appearance of the normal and the changed No. 14 is seen in Fig. 1, in which the D group shows chromosomes from two diploid cells and a tetraploid cell from three different

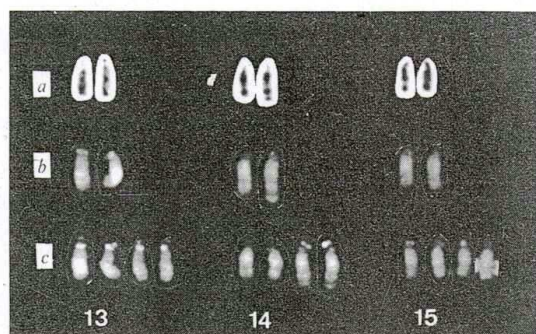


Fig. 1 Appearance of normal and changed chromosome No. 14. See text.

tumours. Fig. 1a is from a cell stained with Giemsa, after specific pretreatment giving a pattern similar to the fluorescence pattern (our unpublished results); b and c are from cells stained with quinacrine mustard (QM) using a modification of the original technique of Caspersson *et al.*². In each cell the normal chromosome is to the left and the homologue with the extra band to the right, and in Fig. 1c each homologue is represented by two chromosomes. Giemsa and QM staining shows in the changed homologue an extra band at the end of the long arm, similar to the normal fluorescing bright band near the end of the long arm (band q7¹). This extra band often appears a little less luminescent, more weakly stained with Giemsa, and somewhat narrower than the normal q7. It is impossible to decide whether the new band represents a duplication of a segment within No. 14, as an inverted duplication of the distal segment, or a translocation of a segment from another chromosome.

Table 1 Extra Marker Band at the End of the Long Arm of Chromosome No. 14 in Burkitt Lymphomas

Patient No.	Sex	Biopsies	Cultures
1	Male		+
2	Male		+
3	Male		+
4	Male		+
5	Female		+
6	Female	+	
7	Male	+	
8	Male	+	
9	Male	+	+
10	Female	+	+
11	Male		-
12	Male	-	-

+, Extra band present; (-) no extra band.

During the work we found many other markers, including the chromosome No. 10 with a secondary constriction, well known in several culture cell lines of Burkitt tumours. None of these markers, however, have been found as consistently and in so high a proportion of tumours, as the one described in No. 14. As this additional band was found in all cells in a high proportion of the Burkitt tumours examined, both in biopsies and in cultures, and as it was visible not only in QM-stained slides by fluorescence microscopy but also in Giemsa-stained slides by light microscopy, we consider it a reliable marker. The fact that a low proportion of the tumours examined did not have the marker band may mean that it had disappeared during the development of the tumour, or that there are two types of Burkitt tumours, perhaps representing different clinical entities, one with the additional band, and the other without.

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GEORGE MANOLOV
YANKA MANOLOVA

Institute of Genetics,
University of Lund,
S-223 62 Lund

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Sea Urchin Eggs Release Protease Activity at Fertilization

At fertilization, sea urchin eggs release macromolecules into the surrounding seawater. The release of these molecules (fertilization product¹) results from the lysis of the peripheral layer of cortical granules^{2,3} and possibly from other sites on the cell surface. Three macromolecules of the fertilization product have been identified: (1) a β -1,3-glucanohydrolase⁴, (2) a protein that forms part of the fertilization membrane⁵ and (3) hyalin⁶, the major constituent of an extracellular gel that surrounds the developing embryo⁷.

We now report the presence of a trypsin-like protease in the fertilization product released from fertilized or parthenogenetically-activated eggs. The activity is assayed radio-metrically (in seawater at pH 8.0) by the release of trichloroacetic acid (TCA)-soluble radioactivity from a ¹⁴C-labelled protein or colorimetrically, using the synthetic substrates α -N-benzoyl-L-arginine ethyl ester, HCl (BAEE)⁸ and *p*-toluenesulphonyl-L-arginine methyl ester (TAME)⁹. The enzymatic activity is completely inhibited by soybean trypsin inhibitor (SBTI). If SBTI is present in the seawater during fertilization the elevation of the fertilization membrane is severely impaired, suggesting that the protease may function in the elevation of the fertilization membrane.

Strongylocentrotus purpuratus gametes were shed by pouring 0.5 M KCl into opened body cavities. Egg jelly was removed by extensive washing and agitation in filtered seawater and each ml. of packed cells resuspended in 7 ml. seawater containing 0.02 M Tris (pH 8.0). Undiluted semen (25 μ l.) was added per 8 ml. egg suspension (15° C) and 5 min later the eggs were sedimented by hand centrifugation. The supernatant seawater was removed and centrifuged at 16,000g for 10 min. The clear supernatant, here designated fertilization product and containing those materials that are released by the egg and pass through the fertilization membrane, was used as the standard enzyme preparation and contained an average of 0.22 mg/ml. protein.

A radioactive protein substrate was prepared from 5 ml. (packed volume) of 20 h sea urchin blastulae incubated for 4 h at 15° C in 200 ml. seawater with 0.5 μ Ci/ml. ¹⁴C-protein hydrolysate (Schwarz). The blastulae were collected by centrifugation, extracted four times with 10% TCA (the second extraction was at 90° C for 20 min), four times in chloroform-methanol (3:1), three times in 95% ethanol and then dissolved in 20 ml. 0.05 N NaOH. The solution contained 8 mg protein/ml. (Lowry assay) and the specific activity was 224,000 d.p.m./mg. One ml. aliquots of the stock solution of radioactive protein were stored at -10° C.

For the assay, a 1 ml. portion of the radioactive substrate was thawed and the protein precipitated by adding 1 ml. 10% TCA. The precipitate was sedimented in a clinical centrifuge and dissolved in 3 ml. seawater containing 0.02 M Tris buffer,