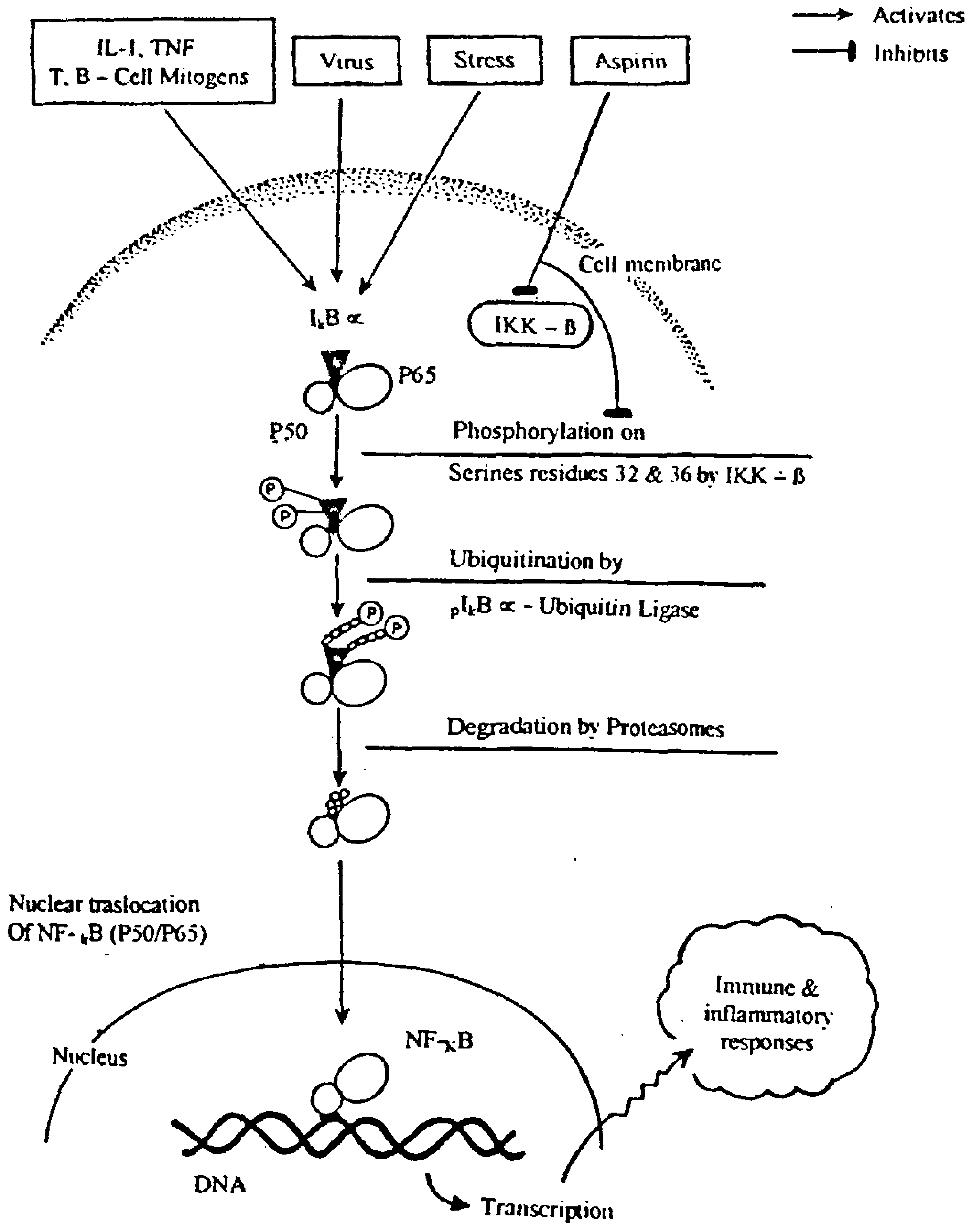

Figure 1.

yotes (but not in bacteria, so the name exaggerates somewhat), which marks other proteins for degradation by the proteasome system[3] – to the phosphorylated NF-$_{\kappa}$B inhibitor I$_{\kappa}$Bα as shown in Figure 1 (ref. 4).

On release, NF-$_{\kappa}$B rapidly moves to the nucleus where it binds specific DNA sequences, promoting the transcription of genes that influence defence mechanisms such as inflammatory and immune responses. Thus, if IKK-β is inhibited by aspirin, nuclear localization of NF-$_{\kappa}$B and subsequent transcription is blocked.

It also has been shown that several other kinases, including the homologous and functionally related IKK-α, are not affected by aspirin. The problem with using high doses of aspirin is that it has virtually no therapeutic window, that is the dose at which aspirin gives relief from chronic rheumatic disease is very close to the dose that generates side effects, including headaches, dizziness and tinnitus. However, a hypothesis has been developed wherein IKK-β is only one of the several targets for high doses of aspirin, the unwanted side-effects resulting from activity against other targets[5].

Although there is a broad consensus that aspirin relieves many of the symptoms associated with inflammatory diseases, it does not prevent the hallmark of progressively crippling rheumatoid arthritis – the destruction of joints. This could be due to the limited effect that aspirin can have on NF-$_{\kappa}$B activation, constrained as it is by the narrow therapeutic window. In light of such findings, we may be able to generate potent molecules that can prevent rheumatoid joint destruction, rather than just relieving the symptoms associated with that destruction. Until then, take two aspirins and get relief in the morning.

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Unravelling the biosynthesis of vitamin C in plants

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The importance of vitamin C to human health is well established. Vitamin C, a water soluble vitamin, is chemically defined as ascorbic acid. Ascorbate mainly acts as an antioxidant and has a role in collagen biosynthesis, the deficiency of which results in scurvy[1]. Animals produce ascorbic acid from glucose via D-glucuronate and L-gulono-1,4-lactone (Figure 1). D-glucuronate is at first reduced to L-gulonate which in turn undergoes dehydration to produce L-gulono-1,4-lactone. L-gulono-1,4-lactone is oxidized to ascorbic acid by a microsomal L-gulono-1,4-lactone oxidase (EC

1.1.3.8). The carbon at the C-1 position of glucose appears at the C-6 position in ascorbic acid. The inversion of the hexose skeleton occurs after glucuronate formation. Humans and other primates require ascorbate in their diet because of loss of the functional form of the last enzyme, L-gulono-1,4-lactone oxidase, of the biosynthetic pathway[1].

Ascorbate is present in millimolar concentrations in plants. Its role in the plant autoxidation system, photosynthesis, transmembrane electron transport and possibly cell expansion, is now being recognized[2]. Despite the multifarious functions of ascorbic acid in plants, its biosynthesis in plants was poorly understood. Concerted efforts by Smirnoff and his group have recently led to the unravelling of the biosynthetic pathway of L-ascorbic acid in plants[3]. Two pathways for ascorbic acid biosynthesis have been proposed – one analogous to the animal pathway, and the second chemically distinct[2]. The sole biochemical evidence for the animal or inversion pathway in higher plants was the identification and isolation of a cDNA encoding L-galactono-1,4-lactone dehydrogenase enzyme, catalysing the oxidation of L-galactono-1,4-lactone to ascorbic acid[4,5]. L-galactono-1,4-lactone dehydrogenase was found to be an intrinsic protein located at the mitochondrial inner membrane[6]. The production of L-galactono-1,4-lactone from D-galacturonic acid requires inversion of the hexose carbon skeleton. Tracer studies, however, indicated that inversion of the glucose carbon skeleton does not occur during ascorbic acid biosynthesis in higher plants[2]. Loewus[7] proposed an alternative non-inversion pathway based on tracer incorporation and isotope dilution experiments, involving D-glucosone and L-sorbosone as intermediates. ^{14}C labelled glucosone and sorbosone were incorporated into ascorbic acid in bean and spinach leaf. In pea embryonic axes, though the incorporation of glucosone was 7 times more when compared to that of D-glucose, the incorporation of label into ascorbic acid from D-glucose and L-sorbosone was more or less the same[8]. Furthermore, the enzyme that converts D-glucose to D-glucosone is not detected in plants, whereas, a dehydrogenase enzyme catalysing the transformation of L-sorbosone to ascorbic acid could be isolated[7]. These evidences for the non-inversion pathway were, however, inconclusive.

The available literature indicated that during transformation of D-glucose to ascorbate in plants, there is conservation of the hydroxymethyl group at C-6, an epimerization at C-5 and no inversion of the carbon chain. Smirnoff *et al.*[3] proposed a pathway (Figure 2) in which L-galactono-1,4-lactone is produced from D-glucose and D-mannose through GDP-D-mannose, GDP-L-galactose, L-galactose and L-galactose-1,4-lactone, and is consistent with the previous findings. D-mannose and L-galactose were found to be effective precursors for ascorbate synthesis when compared to D-glucose. A new enzyme catalysing oxidation of L-galactose to L-galactono-1,4-lactone, referred to as L-galactose dehydrogenase, was identified from pea and *Arabidopsis thaliana* leaves[3]. The enzyme also oxidized L-sorbosone, but with very low affinity. Thus the L-sorbosone dehydrogenase activity previously reported could be that of the L-galactose dehydrogenase. The osones are, therefore, unlikely to be the intermediates of ascorbic acid biosynthesis.

The major form of galactose in plants is D-galactose. L-galactose is formed by hydrolysis of GDP-L-galactose, which in turn is derived from GDP-D-mannose. GDP-D-mannose-3,5-epimerase has been recently detected in extracts of pea embryonic axis and *A. thaliana* leaves using labelled GDP-D-(U-^{14}C) mannose[3]. To date, mutant alleles representing four different loci (vtc 1, vtc 2, vtc 3, and

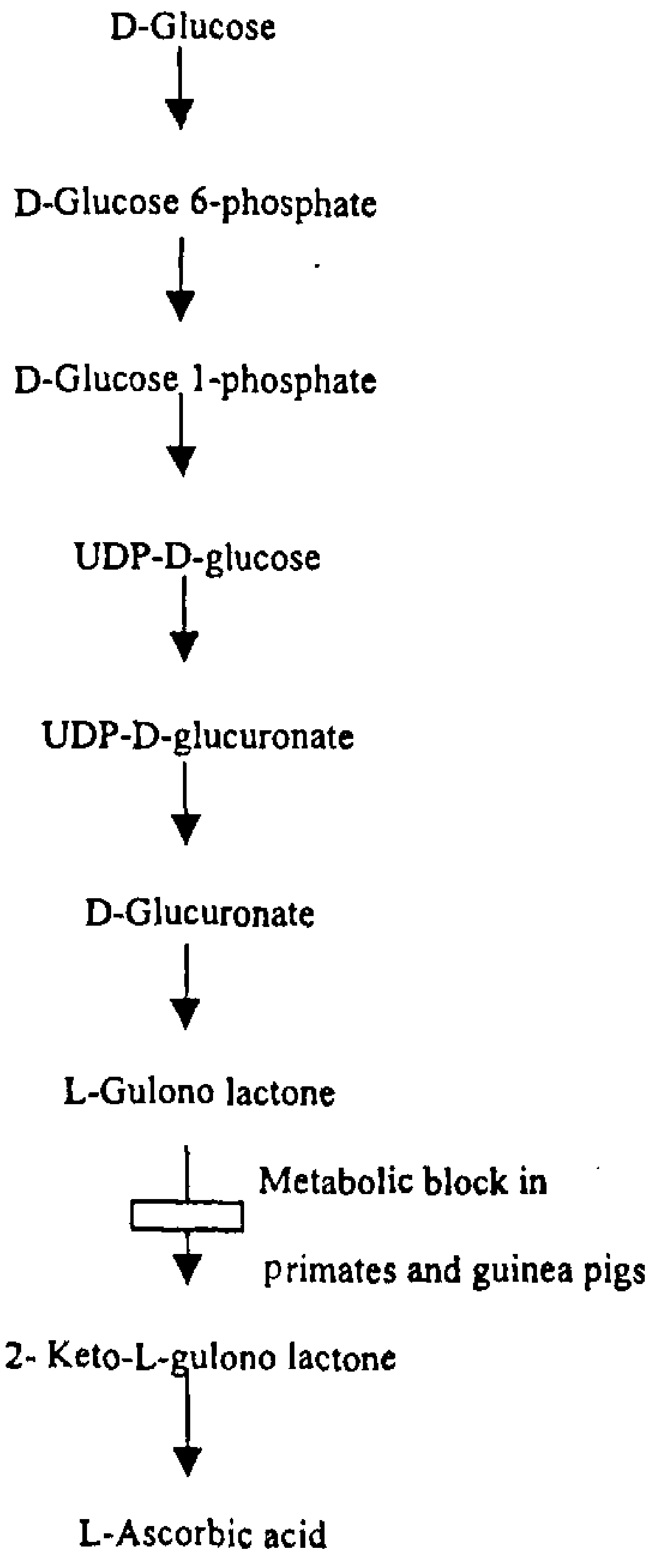

Figure 1. Inversion or the uronic acid pathway for the synthesis of L-ascorbic acid in animals.

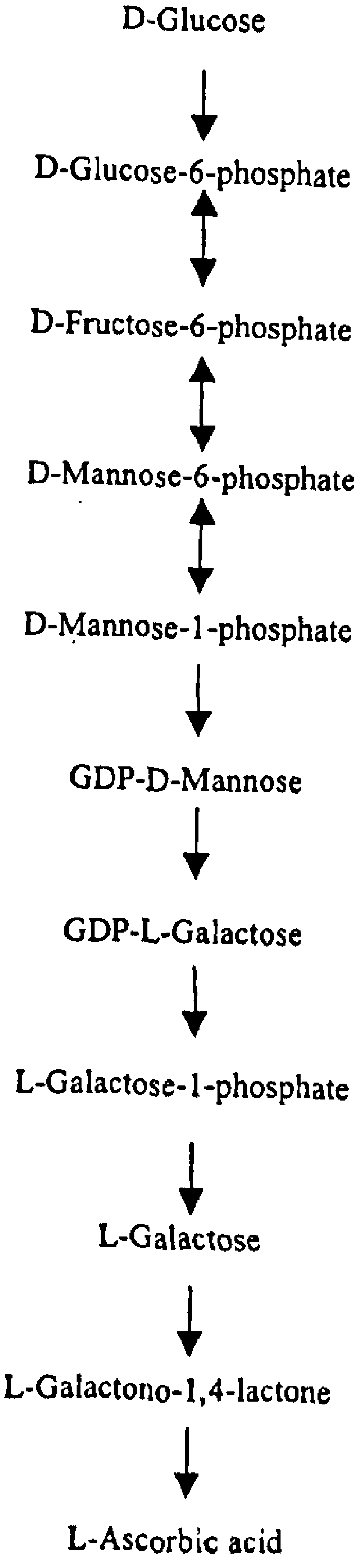

Figure 2. Newly discovered pathway for the biosynthesis of L-ascorbic acid in plants.

vtc 4) involved in maintaining the concentration of ascorbic acid have been isolated[9]. L-ascorbic acid deficient and ozone sensitive mutant vtc 1 (ref. 10) has recently been shown to be defective in the enzyme GDP-D-mannose pyrophosphorylase[11]. All these evidences are in favour of the pathway proposed by Smirnoff as the sole route of L-ascorbic acid synthesis in higher plants. However, *Arabidopsis* cell suspension cultures have been recently shown to synthesize and accumulate L-ascorbic acid from a number of precursors, viz. L-galactose, L-glucose, galacturonic acid methyl ester, glucuronic acid methyl ester, L-gulono-1,4-lactone, and D-glucurono-lactone[12]. Surprisingly, galacturonic acid methyl ester was found to be at least as efficient a substrate as L-galactono-1,4-lactone. Though the results supported L-galactose based synthesis of L-ascorbic acid (Smirnoff pathway), the ability of the plants to efficiently synthesize L-ascorbic acid from L-galacturonic acid methyl ester could not be ruled out. L-gulono-1,4-lactone has recently been identified in plant extracts and a NADPH-dependent enzyme catalysing the reduction of D-glucuronolactone, is being characterized[12]. Thus plants can synthesize L-ascorbic acid both from L-galactose and galacturonic acid. The unravelling of the ascorbate biosynthesis in higher plants has filled a major gap in plant carbohydrate metabolism, as up to 10% of soluble carbohydrate content of leaves can be L-ascorbate[3].

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RESEARCH SNIPPETS

Haze and crop yields

'Case study of the effects of atmospheric aerosols and regional haze on agriculture: An opportunity to enhance crop yields in China through emission controls'
W. L. Chameides *et al.*
Proc. Natl. Acad. Sci. USA, 1999, **96**, 13626–13633

Atmospheric aerosols affect the flux of solar radiation passing through the atmosphere. This study suggests 'that regional haze in China is currently depressing optimal yields of ~ 70% of the crops by at least 5–30%'. Reducing the severity of regional haze through air pollution control could preferentially result in a significant increase in crop yields and help meet growing food demands in coming decades.

Structure of a polio virus–receptor complex

'Three-dimensional structure of polio virus receptor bound to polio virus'
D. M. Belnap *et al.*
Proc. Natl. Acad. Sci. USA, 2000, **97**, 73–78

'Interaction of the polio virus receptor with polio virus'
Y. He *et al.*
Proc. Natl. Acad. Sci. USA, 2000, **97**, 79–84

Two independent determinations of the structure of the polio virus bound to its cellular receptor (Pvr) have been achieved at 21–22 Å resolution by a combination of cryoelectron microscopy and image reconstruction. The receptor, Pvr (CD 155), is a member of the IgG superfamily and acts as an 'unzipper' that initiates changes in the virion that prime it for uncoating.