may well be of importance in understanding the aetiology of cancer.

Evidence suggesting a relationship between lysosomal damage, produced by selective photosensitization, and the occurrence of chromosome aberrations has already been presented (Allison & Paton, 1965). It was suggested that released lysosomal deoxyribonuclease might be able to penetrate the nucleus and attack chromosomes. We have shown that highly purified lysosomal deoxyribonuclease can produce breaks when incubated with isolated polytene chromosomes of *Chironomus*, in keeping with results of other workers with pancreatic deoxyribonuclease. Thus DNA strands, accessible to enzyme, play an important part in maintaining the linear integrity of interphase chromosomes. To obtain further evidence in support of a role of enzymes in chromosome breakage, a method has been devised for introducing deoxyribonuclease into mammalian diploid cells and observing its effects. When the enzyme is added to cells in the presence of 1·5M-$MgSO_4$ and the cells are incubated in normal medium for 24hr., numerous chromatid breaks are found; these are significantly commoner than in control cells treated with $MgSO_4$ alone. If cells are exposed to enzyme in normal medium, or iso-osmotic or hyperosmotic NaCl, no effects are observed, and it is supposed that the $MgSO_4$ facilitates entry of the enzyme into cells or prevents breakdown of the enzyme. Pancreatic deoxyribonuclease or highly purified lysosomal deoxyribonuclease (kindly provided by Dr G. Bernardi) are both effective, and chromatid breakage has been found in all cell systems studied (W1-38 human diploid cells, mouse-embryo cells and Chinese-hamster cells). Selective inhibitors of deoxyribonuclease, double-stranded polyribonucleotides with anti-parallel chains, inhibit the chromatid-breaking effect. Lysosomal stabilizers potentiate the chromosome breakage, which implies that the effect is due to introduced rather than endogenous enzyme. As the quantity of enzyme is increased, the number of breaks rises but the proportion of affected cells does not increase above a plateau value. This suggests that the chromosomes may be vulnerable to enzymic attack only at a certain phase of the division cycle, presumably when DNA is being replicated. The relationship of these results to recent ideas on the role of an endonuclease in DNA replication will be discussed.

Another point of interest is the uptake of polybenzenoid hydrocarbon carcinogens by lysosomes (Allison & Mallucci, 1964), which has been shown to be due to binding by a specific glycolipid constituent of lysosomes (Barrett & Dingle, 1967). Although this is probably responsible for some cytotoxic effects of certain carcinogens (Allison & Dingle, 1966) it is not yet clear how it is related to carcinogenesis. Evidence has also been presented that particles such as asbestos or silica (which can under certain conditions be carcinogenic) accumulate in lysosomes, and that damage to lysosomal membranes contributes to their cytotoxic effects (Allison, Harington & Birbeck, 1966).

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Lysosomes and Congenital Malformations

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Although a large number of chemically diverse compounds have been found to cause congenital malformations in laboratory animals (see Kalter, 1968), very little is known about the molecular basis of teratogenesis. This paper reviews the evidence that certain agents may owe their embryopathic activity to a primary action on lysosomes. A more extensive survey of much of the material has already been published (Lloyd & Beck, 1969).

Acidic bisazo-dyes, typified by Trypan Blue, are powerful teratogens in several species, and a wealth of information is available on many aspects, particularly with respect to rodents (Beck & Lloyd, 1966; Kalter, 1968). Early studies (Wilson, Beaudoin & Free, 1959) showed that, after injection of Trypan Blue into a pregnant rat, the dye does not enter the embryonic cells in detectable amount but is heavily concentrated in the intensely phagocytic epithelial cells of the yolk-sac. Within these cells the dye is found in lysosomes (Lloyd, Beck, Griffiths & Parry, 1968). Two major theories exist to explain the teratogenic action of Trypan Blue in rodents, both owing their origins to Dr J. G. Wilson. The first (Wilson *et al.* 1959) proposes a direct action of the dye on the developing embryo. In the absence of evidence that Trypan Blue enters the embryonic cells, a surface action must be assumed. Rat embryos are susceptible to Trypan Blue teratogenesis during a very restricted period of development, and Wilson *et al.* (1959) pointed out that this corresponds to the period before the embryo is 'surrounded' by the yolk-sac. On this theory the vacuolar system of the yolk-sac serves

a protective role by immobilizing Trypan Blue and preventing its access to the later embryo. An alternative theory is that Trypan Blue affects embryonic development by deranging the transport functions of the yolk-sac. Originally proposed by Wilson, Shepard & Gennaro (1963), this theory has been developed by the present authors. The rat yolk-sac has been found active in intralysosomal digestion of endocytosed proteins (Beck & Lloyd, 1968; Williams, Lloyd & Beck, 1969) and a role for this process in embryotrophic nutrition has been proposed (Beck & Lloyd, 1968). The observation that Trypan Blue is an inhibitor of several lysosomal enzymes of rat yolk-sac led us to suggest that Trypan Blue may cause embryopathy by denying the embryo metabolites normally provided by the digestive activity of yolk-sac lysosomes (Beck, Lloyd & Griffiths, 1967; Lloyd & Beck, 1968). Support for the view that Trypan Blue is an intralysosomal enzyme inhibitor has come from the histochemical studies by Greenhouse, Pesetsky & Hamburgh (1969) and from biochemical observations of Trypan Blue-laden liver lysosomes (Davies, Lloyd & Beck, 1969*a*).

The trypanocide suramin has many features in common with Trypan Blue. Chemically similar, it is taken into rat liver lysosomes (P. J. Jacques, quoted by Allison, 1968) and is an inhibitor of some lysosomal enzymes in rat liver (M. Davies, personal communication). Rat liver lysosomes containing suramin have a decreased digestive capacity (Davies, Lloyd & Beck, 1969*b*). Unpublished work by the present authors demonstrated an embryolethal effect of suramin in the absence of maternal death. Like Trypan Blue, suramin does not appear to penetrate the embryo and is found in highest concentration in the yolk-sac (R. L. Schultz, personal communication).

Sodium aurothiomalate is an inhibitor of several lysosomal enzymes *in vitro* (Persellin & Ziff, 1966; Ennis, Granda & Posner, 1968), and liver lysosomes from rats injected with the drug show a decreased digestive capacity and an increased susceptibility to rupture (Davies *et al.* 1969*b*). Norton, Lewis & Ziff (1968) have described electron-dense deposits in lysosomes from human and rabbit tissues after injection with the drug. Similar profiles have been seen in yolk-sac lysosomes from treated pregnant rats and the drug has been found to cause embryonic death and malformations in rats at doses well below the maternal LD_{50} (J. B. Lloyd & F. Beck, unpublished work).

Triton WR-1339, a non-ionic detergent, has been found to be embryotoxic in rats by Schultz & Schultz (1966) and teratogenic in mice, rats and rabbits by Tuchmann-Duplessis & Mercier-Parot (1964). The two activities are apparently separable and possibly do not operate by the same mechanism (Roussel & Tuchmann-Duplessis, 1968). Triton WR-1339 and some homologous molecules are active against the tubercle bacillus *in vivo* but not *in vitro*, and Allison (1968) has proposed that the detergent may modify the intralysosomal environment so that it is unsuitable for bacterial multiplication. No details of the precise changes caused are available, although Hart, Gordon & Jacques (1969) have shown that liver lysosomes from rats pre-injected with the detergent contain an antituberculous material, which is lipid in nature. After injection into pregnant rats Triton WR-1339 does not seem to enter the embryo, but is taken into the lysosomes of the yolk-sac epithelial cells, where it causes lysosomal swelling (Schultz, Reger & Schultz, 1966), similar to that seen in liver (Wattiaux, Wibo & Baudhuin, 1963). A teratogenic action dependent on a primary action on lysosomes is made more plausible by the demonstration (H. Tuchmann-Duplessis, personal communication) that cortisone, a lysosome stabilizer, greatly decreases the teratogenic activity of Triton WR-1339 in mice.

There remain a number of teratogens, for which a lysosomal mechanism of action may be suspected on the basis of effects in other biological systems, but where little or no direct evidence is available. Excess of vitamin A is teratogenic (see Kalter, 1968) and causes tissue necrosis in some systems, apparently by stimulating exocytosis of lysosomal enzymes (Roels, 1969). A plausible mechanism of teratogenesis could be envisaged on this basis. However, in a biochemical study R. L. Schultz (personal communication) was not able to detect any changes in lysosome stability in 12-day rat embryo, yolk-sac or placenta after injection of the mothers with teratogenic doses of vitamin A, and it is as well to remember that vitamin A has actions on biological membranes other than those of lysosomes (Dingle & Lucy, 1965). Among the teratogenic hypovitaminoses (see Kalter, 1968) are two (vitamin A and E deficiencies) where effects on lysosome stability have been described (see Roels, 1969). Cortisone induces cleft palate in mice (Kalter & Warkany, 1959) and is a well-known lysosome stabilizer (Weissman, 1969). Dimethyl sulphoxide causes congenital malformations in golden hamsters (Ferm, 1966) and has been claimed to affect the permeability of the lysosome membrane (Misch & Misch, 1969). Lysosomes are involved in the uncoating and cytopathic effects of viruses (see Allison, 1967) and this may be important in considering the mode of action of teratogenic viruses.

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Lysosomes and Mucopolysaccharidoses

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A series of conditions are known in human pathology in which there is a tissular accumulation of both mucopolysaccharides and glycolipids. Some of them have been classified as mucopolysaccharidoses, others as gangliosidoses or lipidoses, and they could be adequately designated as 'lipomucopolysaccharidoses'. The clinical syndrome includes variable degrees of dwarfism, hepatosplenomegaly, skeletal deformities and mental retardation (for a more extensive bibliography see Dorfman, 1966; Hers & Van Hoof, 1969). In the Hurler syndrome, there is an excessive urinary excretion of chondroitin sulphate B, heparitin sulphate and keratan sulphate, either alone or in association. In the pseudo-Hurler disease, also called Tay–Sachs disease with visceral involvement or generalized gangliosidosis, there is a tissue accumulation of the GM_1 ganglioside, which bears a terminal galactosyl unit, and of a mucopolysaccharide rich in galactose. Several other clinical types of mucopolysaccharidoses have also been reported.

As storage diseases in which the depot is chemically heterogeneous, the mucopolysaccharidoses must be regarded as potential inborn lysosomal diseases. This group of affections (Hers, 1965) includes all conditions resulting from the congenital abnormality of one lysosomal protein; when this protein is an enzyme, the loss of its activity causes the intravacuolar storage of all the compounds that would normally require the missing enzyme for their degradation in the process of either autophagy or of heterophagy (for a review on the physiology of lysosomes, see de Duve & Wattiaux, 1966). Like other digestive enzymes, the lysosomal acid hydrolases usually do not display a high substrate specificity and most of them are able to open one type of linkage, whatever the molecule in which it is present; therefore the loss of their activity easily explains the chemical heterogeneity of the depot that is so characteristic of many mucopolysaccharidoses and lipidoses. As a rule, the inborn lysosomal diseases are progressive and affect most tissues of the organism. Mechanical disturbance due to the lysosomal enlargement, discharge of lysosomal enzymes in the cytoplasm or dysfunction of the lysosome itself may be the cause of the pathological manifestation.

The involvement of the lysosomes in the pathogeny of mucopolysaccharidoses is clearly indicated by the ultrastructure of the tissues. In the liver of both Hurler (Van Hoof & Hers, 1964) and pseudo-