view of the evidence from experiments with bacteria, that the hormone could act directly to de-repress the genes and more likely that the hormone interacts with a protein that is itself the genetic repressor. No unequivocal evidence for the existence of such a repressor is yet available in eukaryotic cells, but the protein receptors for steroid hormones and the histones are among the possibilities that have been suggested.

Many hormones stimulate the production of proteins already synthesized by the organism. In some instances, for example insulin and growth hormone, the evidence shows that the anabolic response is not dependent on prior RNA synthesis, but that control of the translational processes is involved (Wool *et al.* 1968; Korner, 1968). It is known that diabetes results in a defect of the large ribosomal subunit of muscle, whereas hypophysectomy results in a defect in small ribosomal subunit of liver.

Corticosteroids can induce the synthesis of enzymes by an actinomycin-sensitive process, and this has been interpreted by several workers as indicating an action, direct or indirect, of the hormone on the genome (Kidson & Kirby, 1964), but this view is opposed by others (for review see Korner, 1967). The abolition of a response to a hormone by actinomycin is not, however, unequivocal evidence of the need for RNA synthesis, since the antibiotic has actions other than abolition of DNA-directed RNA synthesis. Tomkins *et al.* (1966) have argued, on the basis of the phenomenon of superinduction of enzymes by actinomycin, that regulator genes exist producing substances that inactivate the messengers for the enzymes, but this view has been challenged (Reel & Kenney, 1968). Corticosteroids, insulin and glucagon all induce

tyrosine transaminase in rat liver and in cells in tissue culture, but each hormone uses a different mechanism to achieve this.

Many of the hormones stimulate RNA synthesis and the activity of the RNA polymerase enzyme. In some cases (e.g. oestrogens and androgens) the evidence (Nicolette, Lemahieu & Mueller, 1968) suggests that this is a necessary prerequisite for the protein anabolic responses to these hormones, but in other cases (e.g. insulin and growth hormone) it appears to be secondary to the stimulation of protein synthesis.

There is no doubt that the long-term application of many hormones results in the synthesis of more ribosomes and endoplasmic reticulum. Tata (1968) is among those who have argued that this change in the cytoskeleton is of importance in the protein anabolic action of thyroid and other hormones.

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COLLOQUIUM ON 'LYSOSOMES AND DISEASE'

Lysosomes, Chromosomes and Cancer

By A. C. ALLISON and GILLIAN R. PATON. (*Clinical Research Centre Laboratories, National Institute for Medical Research, Mill Hill, London N.W.7*)

It is widely, although not universally, believed that the basic defect in cancer is a chromosomal mutation in somatic cells. This could allow deletion of genetic information (for instance that controlling growth) without affecting the capacity of cells to divide. Alternatively the chromosomal mutation could allow acquisition of new genetic information, e.g. by incorporation of a viral genome or by somatic recombination with gene conversion. The new information could be expressed as antigens not present in parental cells or in other ways. Examples of suggestive relationships between chromosomal

aberrations and cancer include the presence of the Ph¹ chromosome (with a partial deletion of the small chromosome 21) in chronic myeloid leukaemia. Children with Bloom's syndrome, Fanconi's anaemia and ataxia telangiectasia have a greatly increased tendency to develop malignancy. When their skin cells or blood leucocytes are cultured *in vitro* numerous chromosome aberrations are found (see German, 1969). Individuals homozygous for the recessive gene giving rise to xeroderma pigmentosum have a much increased risk of developing malignant changes where they are exposed to ultraviolet light. Evidence has been presented that the cells of these patients lack a mechanism that can repair damage produced by ultraviolet radiation (Cleaver, 1968). Hence further study of the mechanisms of chromosome breakage and repair

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-MgSO₄ 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 MgSO₄ 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 MgSO₄ 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 (WI-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 polycyclic aromatic 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

By J. B. LLOYD and F. BECK. [*Department of Biochemistry, University College, Cardiff, and Department of Anatomy and Histology, London Hospital Medical College (University of London), London E.1*]

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