rats. The amount of ^{14}C-labelled lipid derived from added glucose arising from the Embden-Meyerhof pathway has been calculated by the method described by ABRAHAM *et al.*[3]. While $85 \pm 6\%$ arose from glycolysis in control experiments this value fell to $58 \pm 6\%$ in experiments in which prolactin was added to the tissue slices (Fisher's $P = 0.023$). By difference it is apparent that the contribution of the non-glycolytic route is more than doubled in the presence of prolactin.

In contrast with the above results prolactin had no consistent effect *in vitro* on the metabolism of specifically labelled glucoses by mammary-gland slices from lactating rats (11th–18th day).

WINEGRAD *et al.*[4] have recently shown that prolactin *in vitro*, in concentrations similar to those used in the present study, increased both the oxidation of glucose carbon to $^{14}CO_2$ and the incorporation of glucose carbon into fatty acids by adipose tissue from normal-fed rats. The present results are also in accord with those of ABRAHAM *et al.*[5] who have investigated the oxidation of glucose and acetate and lipogenesis from these substrates by mammary glands of hypophysectomised rats in which lactation was hormonally induced. These authors found that administration of prolactin and Δ^1-hydrocortisone acetate together brought about pronounced changes in the pattern of glucose metabolism, a greater proportion of the glucose-derived fatty acids arising from the pentose phosphate pathway.

It is of interest that the effect of prolactin disappears with the onset of lactation while the action of insulin is considerably enhanced[6], a point of contrast which suggests that these two hormones have different mechanisms of action although the general pattern, the parallel increase in the oxidation of glucose and synthesis of lipid, is apparent in both cases.

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The mode of action of actinomycin D

Actinomycin D is one of a series of polypeptide antibiotics, the first of which was reported by WAKSMAN AND WOODRUFF in 1940[1]. It has powerful bacteriostatic effects on many micro-organisms, particularly Gram-positive bacteria.

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; d.ATP, deoxyadenosine triphosphate; d.GTP, deoxyguanosine triphosphate; d.TPP, thymidine triphosphate; d.CTP, deoxycytosine triphosphate; Tris, tris (hydroxymethyl)aminomethane.

At concentrations of 0.25–0.75 μg/ml it completely inhibits growth of *Staphylococcus aureus* strain Duncan. Respiration and glycolysis of washed suspensions of this organism are unaffected by concentrations of up to 100 μg/ml, but in a synthetic medium[2], containing amino acids, purines, pyrimidines and glucose, the net synthesis of protein, inducible β-galactosidase, and nucleic acids is inhibited at growth-inhibitory concentrations. The extent of the inhibition obtained with a given concentration of the antibiotic is dependent on the density of the cell suspension used.

Addition of actinomycin D to an exponentially-growing culture of *Staph. aureus* produces an immediate inhibition of the synthesis of RNA, followed rapidly by inhibition of protein synthesis. Inhibition of DNA synthesis occurs after a lag period of some 10–15 min, but the degree of inhibition obtained for a given concentration of actinomycin D is less than that occurring for RNA or protein synthesis. Actinomycin D also inhibits the incorporation of [^{14}C]labelled amino acids, purines or pyrimidines into the hot-trichloroacetic acid-insoluble and -soluble fractions, the effects correlating with those on net protein and nucleic acid synthesis. Incorporation of [^{14}C]glutamic acid into the cell wall material of *Staph. aureus*, isolated as described by PARK AND HANCOCK[3], is however unaffected by actinomycin D at concentrations of up to 20 μg/ml. The inhibitory action of actinomycin D on the incorporation of ^{14}C-labelled substances into the "protein" and "nucleic acid" fractions is antagonised by the addition of a preparation of staphylococcal nucleic acid to the incubation mixture. Fractionation and purification of the nucleic acid indicates that the antagonism is entirely due to the DNA fraction. DNA preparations from calf thymus, wheat germ, herring roe, *Brucella* and *Bacillus megaterium* also have this antagonistic effect.

The addition of DNA to a solution of actinomycin D at pH 7.0 reduces the absorption of the latter, and shifts the position of the absorption maximum towards the red end of the spectrum. The complex formed appears to be freely dissociable. Equilibrium dialysis[4] indicates that the absorption maximum of the actinomycin-DNA complex is about 465 mμ, and that, in a solution containing 136 μg/ml actinomycin D and 600 μg/ml DNA at pH 7.0, 84% of the actinomycin is in the complexed form. Actinomycinic acid, the dicarboxylic acid derived from actinomycin D, is without antibacterial action, and the addition of DNA has no effect on its absorption spectrum. Bacterial RNA (prepared as described in ref. 5, 6), heat-denatured DNA, and heparin have no effect on the biological activity or on the absorption spectrum of actinomycin D.

Investigation of the possible metabolic consequences of the reaction between actinomycin D and DNA indicates that (i) the antibiotic has no significant inhibitory effect on the polynucleotide phosphorylase of *Staphylococcus aureus* in the system described by GRUNBERG-MANAGO, ORTIZ AND OCHOA[7]; (ii) as shown in Fig. 1, actinomycin D inhibits the incorporation of radioactivity into the $HClO_4$-insoluble fraction when [^{32}P]d.ATP is incubated with a crude preparation of the DNA "polymerase" enzyme isolated from *Escherichia coli* in an assay system similar to that described by LEHMAN, BESSMAN, SIMMS AND KORNBERG[8], (iii) actinomycin D inhibits the transformation of *Haemophilus influenzae* Graciae from streptomycin sensitivity to streptomycin resistance when it is added to high concentrations of transforming DNA from a resistant strain. As shown in Fig. 2, at lower DNA concentrations actinomycin has no effect on the percentage of cells transformed compared with the

control. The lack of inhibition at lower DNA concentrations might be due to dissociation of the actinomycin–DNA complex in very dilute solutions.

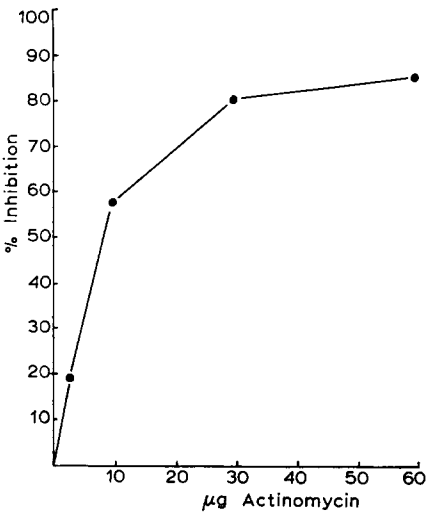

Fig. 1. Inhibition of conversion of [^{32}P]d.ATP into an acid-insoluble product when crude preparation of DNA "polymerase" is incubated at 37° for 60 min. The incubation mixture (0.3 ml) contained 10 μl enzyme preparation, 2 μmoles $MgCl_2$, 20 μmoles glycine buffer, pH 9.2, 0.3 μmole cysteine, 10 μg thymus DNA, about 3.0 μmoles [^{32}P]d.ATP, 6.6 μmoles d.GTP, 2.9 μmoles d.TPP, 4.3 μmoles d.CTP, 5.5 μmoles Tris buffer, pH 7.5 containing 11 μg thymus DNA.

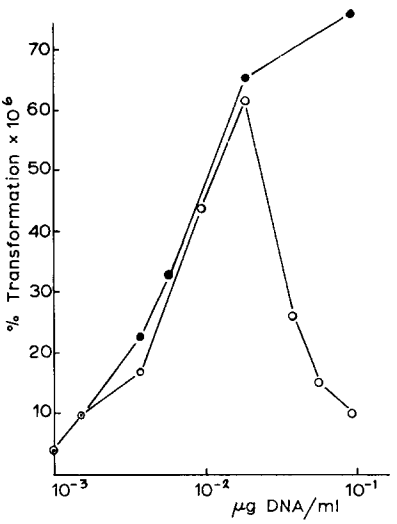

Fig. 2. The effect of actinomycin D on the transformation of *Haemophilus influenzae* from streptomycin sensitivity to streptomycin resistance. ●, in the absence of actinomycin; ○, in the presence of actinomycin at a ratio actinomycin/DNA of 50:1.

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