illustrated by the following model experiment, in which *mono*-2 : 4-dinitrophenyl-cystine is formed from a mixture of cystine and *bis*-2 : 4-dinitrophenyl-cystine.

1 mgm. *bis*-2 : 4-dinitrophenyl-cystine and 10 mgm. cystine were incubated with 2 ml. of 12 *N* hydrochloric acid. After 24 hr. at 37°, 5 ml. water and 10 ml. ether were added and the mixture shaken. Nearly all the yellow colour was found in the aqueous layer, indicating the presence of *mono*-2 : 4-dinitrophenyl-cystine. In a control experiment without cystine, all the colour which was due to *bis*-compound was in the ether layer. The reaction-rate is rapid in strongly acid or in neutral solution and is minimal in dilute acid (about 0·1 *N* hydrochloric acid). This probably explains the fact that insulin is inactivated more readily by heating in neutral than in dilute acid solution[1], if the rearrangement of the —S—S— bridges is assumed to cause inactivation.

The mechanism of the reaction is not clear at present and is under investigation. It may involve the intermediate formation of an —SH compound by reduction or hydrolysis, in which case the actual rearrangement would be brought about by the reaction :

$$-R^1\text{—SH} + R^2\text{—S—S—}R^3 \rightleftharpoons R^2\text{—SH} + R^1\text{—S—S—}R^3.$$

In this case the $R^2$—SH formed would be available to react with a further disulphide, so that only a catalytic amount of an —SH compound could bring about considerable rearrangement[2].

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[1] Dudley, H. W., *Biochem. J.*, **17**, 376 (1923).
[2] Huggins, C., Tapley, D. F., and Jensen, E. V., *Nature*, **167**, 592 (1951).

## Structural Features of Antitumorigenic Corticoids

The antifibromatogenic potency of deoxycorticosterone—prevention of œstrogen-induced abdominal fibroids—diminishes through the substitution $O{=}C_{11}$ (Kendall's compound *A*) and especially through $OH{-}{-}C_{17}$ (Reichstein's compound *S*)[1]. The antifibromatogenic potency of cortisone, which differs from deoxycorticosterone by both these substitutions, is also strikingly diminished ; there were fibroids even with as much as 1,000 μgm. of cortisone acetate per day. But, on the other hand, there apparently was some antifibromatogenic activity of compound *F*, or 17-hydroxy-corticosterone ($OH{-}{-}C_{17}$ and $OH\text{—}C_{11}$); we had thus to raise the question of a 'protective' action of $OH\text{—}C_{11}$ against $OH{-}{-}C_{17}$. However, in view of the variations of the fibrous tumoral effect met with in similar experiments, and especially in view of the small number of experiments performed with compound *F*, the conclusion as to the supposed 'protective' action of $OH\text{—}C_{11}$ against $OH{-}{-}C_{17}$ remained doubtful[1]. In the meantime, we have been able to settle this question in what seems to be a definite manner, thanks to the kindness of Messrs. Merck and Co., who put at our disposal the necessary quantities of compound *F* acetate.

Ten animals (castrated female guinea pigs) were used in the experiment, which lasted 91–92 days. An average of 81 μgm. of œstradiol per day was absorbed from subcutaneously implanted tablets ; 268–447 μgm. (average 350) of compound *F* acetate was absorbed per day from two tablets (a new implantation six weeks after the first one). There were fibroids in nine out of ten animals ; average fibrotumoral effect, 5·2 ; average weight of uterus, 4·6 gm. The quantities of compound *F* were indeed smaller than in some animals of the former series, in which there apparently was prevention ; but there is the fact that fibroids were present in the new series, even with quantities as large as 350–447 μgm. of compound *F* acetate per day. It is thus evident that $OH\text{—}C_{11}$ does not afford 'protection' against $OH{-}{-}C_{17}$, by which antifibromatogenic potency is so greatly diminished (compound *S*).

Although we must drop our original concept of 'protection' by $OH\text{—}C_{11}$ against $OH{-}{-}C_{17}$ with reference to antifibromatogenic corticoids, it is very remarkable that the same concept seems to be valid with reference to the inhibition of the growth of the chick embryo, as evidenced by certain findings of Stock[2]. The effective growth-inhibiting dose of deoxycorticosterone is 250 μgm. ; that of compound *S* ($OH{-}{-}C_{17}$) is as high as 5,000 μgm. ; but the effective dose of compound *F* ($OH{-}{-}C_{17}$ and $OH\text{—}C_{11}$) is only 20 μgm.

Attention may also be directed to a comparative discussion of the structural features of antifibromatogenic and antilymphomatogenic corticoids[3].

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[1] Mardones, E., Iglesias, R., Fuenzalida, F., Bruzzone, S., and Lipschutz, A., *Nature*, **170**, 917 (1952).
[2] Stock, C. C., Ciba Found. Coll. Endocrinol., **1**, 135 (1952).
[3] Lipschutz, A., Mardones, E., Iglesias, R., Fuenzalida, F., and Bruzzone, S., *Science*, **116**, 448 (1952).

## Multi-spots in Paper Chromatograms

During investigations on the synthesis of nucleotides in this laboratory, extensive use has been made of the paper chromatography of phosphorus-containing compounds. In the early experiments it was observed that in a descending pyridine – ethyl acetate – water system, disodium hydrogen phosphate gave two clear spots of approximately equal intensity with $R_F$ 0·17 and 0·55 on spraying with molybdate reagent[1]. Identical spots were obtained with sodium or potassium mono- or di-hydrogen phosphates, whereas ammonium phosphate and free orthophosphoric acid gave only the faster-running spot. Trisodium phosphate showed largely the slower spot with some trailing. (These $R_F$ values are quoted to give an indication of the positions of the spots. The temperature was not strictly controlled.) Other experiments showed the effect was not due to complexes with pyridine as the same double-spot phenomenon occurred in a butanol – water system. By buffering the phosphates with acid and alkali, it was found that the number of spots depended on the *p*H of the solution in which the phosphate was applied to the paper. Potassium dihydrogen phos-