but not significant. The regression relationships were as follows: Stypven time (sec.) $= 50{\cdot}51 + 0{\cdot}80\alpha - 0{\cdot}8\beta$ for the Australians, and $40{\cdot}32 + 0{\cdot}46\alpha - 0{\cdot}09\beta$ for the natives.

The results offer no direct support for a thrombogenic factor in coronary heart disease, but they do suggest that a constituent of alpha-lipoprotein delays clotting in the Stypven test. Whatever may be the nature of this inhibiting factor, there is evidence to suggest that it is not cholesterol itself[3]. In order to confirm these observations further study of the effects of the specific phospholipid and fatty acid constituents of alpha-lipoprotein on the Stypven system will be necessary.

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Effect of Diphosphopyridine Nucleotide on the Inhibition of Rat Liver 3-Hydroxyanthranilic-Acid Oxidase by 2-Acetylaminofluorene and N-2-Fluorenyl-diacetamide

Dunning *et al.*[1] demonstrated that bladder cancer due to 2-acetylaminofluorene was produced in almost all rats (strain Fisher 344) on a diet containing large quantities (1·4–4·3 per cent) of tryptophan. On the other hand, rats on a diet containing smaller amounts of tryptophan did not develop bladder cancer. We showed that the administration of 2-acetylaminofluorene inhibits 3-hydroxyanthranilic-acid oxidase in rat hepatic tissue[2], with consequent accumulation of the two carcinogenic *o*-aminophenolic compounds 3-hydroxyanthranilic acid and 3-hydroxykynurenine[3]. It seemed of interest to pursue these studies in order to elucidate the mechanism of this inhibition.

Kielley[4] has demonstrated that several carcinogenic fluorene compounds inhibit glutamic dehydrogenase; N-2-fluorenyl-diacetamide would therefore act by blocking the utilization of diphosphopyridine nucleotide[5]. In view of this, it seems probable that 2-acetylaminofluorene inhibits 3-hydroxyanthranilic-acid oxidase by affecting the utilization of diphosphopyridine nucleotide (which seems to take part in the reaction[6]). In order to test this hypothesis, we performed new experiments with 2-acetylaminofluorene and N-2-fluoroenyldiacetamide.

Livers, from rats of unspecified strain weighing about 200 gm. and which had received 0·1–0·2 per cent of Hoffmann–La Roche 2-acetylaminofluorene in their diet (Coward) for 2–6 days, were used. The liver was excised, immediately homogenized in the cold in a Potter blendor with Krebs–Ringer phosphate (3 ml. per gm. of tissue), and centrifuged. The supernatant was used as enzyme source.

The incubation mixtures had the following composition: 1 ml. of supernatant; 3,000 μgm. 3-hydroxyanthranilic acid (Hoffmann–La Roche) diphosphopyridine nucleotide (I.S.I.) at a final concentration of 2×10^{-5} *M* (made up to final volume of 6·34 ml. with Ringer's solution). At the same time mixtures without diphosphopyridine nucleotide, without 3-hydroxyanthranilic acid and without tissue were prepared. The mixtures were incubated at 37° C. for 90 min. with frequent shaking.

Quinolinic acid was determined according to the method of Rabinovitz *et al.* with slight modifications[7].

Livers of rats of the same strain, weight and age, which had received the same diet, but without 2-acetylaminofluorene, were assayed for their ability to convert 3-hydroxyanthranilic acid into quinolinic acid under identical experimental conditions.

In one experiment 546 μgm. of quinolinic acid was formed in the presence of normal rat liver (diphosphopyridine nucleotide does not affect this enzymatic activity); 273 μgm. quinolinic acid was formed in the presence of liver from a rat treated with 2-acetylaminofluorene (diphosphopyridine nucleotide added to these incubation mixtures brings the enzymatic activity almost back to normal).

In other experiments we studied the 3-hydroxyanthranilic-acid oxidase activity in rats treated with parenteral injections of N-2-fluorenyl-diacetamide (for which we thank Prof. Kielley and Prof. Weisberger) and the effect of diphosphopyridine nucleotide on this activity *in vitro*. Rats weighing about 200 gm. were treated for four days parenterally with N-2-fluorenyl-diacetamide (40 mgm. in 2 ml. of sterile olive oil per day); on the fifth day the treated animals and the controls, which received 2 ml. of olive oil per day for four days, were killed by decapitation and the 3-hydroxyanthranilic-acid oxidase in the liver determined as described above.

In one experiment 525 μgm. of quinolinic acid was formed in the presence of normal rat liver (diphosphopyridine nucleotide does not potentiate this enzymatic activity); 315 μgm. of quinolinic acid was formed in the presence of liver from a rat treated with N-2-fluorenyldiacetamide (diphosphopyridine nucleotide added to these incubation mixtures brings the enzymatic activity back to normal).

Thus, *in vitro*, diphosphopyridine nucleotide abolished the inhibition of rat liver 3-hydroxyanthranilic-acid oxidase produced by 2-acetylaminofluorene and N-2-fluorenyldiacetamide.

Our work therefore confirms Kielley's[4,5] on the inhibitory influence of fluorene carcinogens on the utilization of the pyridine coenzymes. Moreover, these investigations lead to the following important suggestion: the inhibition of 3-hydroxyanthranilic-acid oxidase is observed both in hepatic tissue of rats bearing Oberling, Guérin and Guérin myeloma at the leukæmic phase[8] and in the hepatic tissue of rats treated with 2-acetylaminofluorene and N-2-fluorenyldiacetamide. In both cases this inhibition is abolished by diphosphopyridine nucleotide.

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