## Conversion of 3-Hydroxyanthranilic Acid to Quinolinic Acid in Presence of Rat Liver treated with 2-Acetylaminofluorene

THE reactions reported here are considered to be the final steps in the chain of reactions whereby tryptophan is converted into vitamin[1]; it shows the inhibitory action of the carcinogenic substance 2-acetylaminofluorene on the reaction 3-hydroxyanthranilic acid→quinolinic acid.

The effect of a high-protein diet on the development of tumours, when 2-acetylaminofluorene is used as the carcinogenic compound, has not as yet been clarified. Morris *et al.*[2] reported that rats maintained on a diet containing 18–24 per cent casein, after administration of 2-acetylaminofluorene, showed a high number of tumours when compared with rats maintained on a 12 per cent casein diet.

Engel *et al.*[3] noticed that the number of weaned rats in which mammary tumours caused by 2-acetylaminofluorene were observed, decreased when casein was increased in the diet. Harris[4] states that the development of liver tumours due to 2-acetylaminofluorene does not depend on the protein-level of the diet.

Dunning *et al.*[5] investigated the connexion between diet and incidence of cancer due to 2-acetylaminofluorene. They found that bladder cancer arose in all rats (Fisher stock 344) which were fed on a diet containing large amounts of tryptophan (1·4–4·3 per cent). Rats fed on a diet containing smaller amounts of tryptophan showed no development of bladder cancer.

The high level of tryptophan in the diet necessary for the development of tumours recalls that the two *o*-aminophenols, that is, 3-hydroxyanthranilic acid and 3-hydroxykynurenine, formed in the degradative metabolism of tryptophan possess carcinogenic activities. Yet if 2-acetylaminofluorene induced a biochemical lesion at a stage when one of the transformation reactions of these two compounds takes place, it would be possible to formulate a hypothesis on the mechanism of the carcinogenic activity of 2-acetylaminofluorene on the bladder, based on the accumulation of one or both of these *o*-aminophenolic derivatives.

Our experiments were conducted on liver of rats maintained on a diet containing 2-acetylaminofluorene of Hoffmann–La Roche (0·1–0·2 per cent in the diet) for periods varying from 2 to 6 days.

Liver taken from rats of the same stock, of similar weight and age, fed on the same diet but without 2-acetylaminofluorene, was tested for its capacity to convert, under the same experimental conditions, 3-hydroxyanthranilic acid (3 mgm.) into quinolinic acid (incubation time always 1 hr.).

The method used for determining quinolinic acid was a slight modification of that of Rabinovitz, Fineberg and Greenberg[6,7].

The results of these experiments clearly demonstrate that, after administration of 2-acetylaminofluorene, the enzymic reaction, that is, the conversion of 3-hydroxyanthranilic acid to *o*-quinoneiminic compound, in liver tissue is highly inhibited; this inhibition results in an average value of about 50 per cent. In one of the experiments 504 μgm. of quinolinic acid was formed in the presence of normal rat liver; 210 μgm. quinolinic acid was formed in the presence of liver of a rat treated with 2-acetylaminofluorene.

The following possible mechanism would explain the carcinogenic activity of 2-acetylaminofluorene: 2-acetylaminofluorene inhibits the conversion of 3-hydroxyanthranilic acid to the *o*-quinoneiminic compound and causes an accumulation of 3-hydroxyanthranilic acid and 3-hydroxykynurenine. These physiological metabolites could in certain conditions lead to tumour formation.

It is important to notice that the reaction of 3-hydroxyanthranilic acid is already inhibited 24 hr. after administration of 2-acetylaminofluorene, while according to Wilson *et al.*[8] tumours arise only after 3–4 months. Therefore the biochemical lesion caused by the carcinogenic compound would appear very early.

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## Demonstration of Antibody in Passive Transplantation Immunity to Crocker Tumour in Mice, using the Schultz–Dale Technique

MITCHISON[1] reported a simple and effective procedure for passively transferring transplantation immunity to a mouse lympho-sarcoma by injecting minced, regional lymph nodes intraperitoneally and draining the grafted areas of actively immunized mice. This phenomenon was later confirmed by Billingham *et al.*[2] using skin homografts. They preferred to call it 'adoptively acquired immunity'. Using the same technique[1], a similar transfer of passive immunity to homologous or heterologous tumour transplantation was conferred upon rats[3,4]. The criteria of immunization adopted in these experiments was the ability of the animal to cause regression of a viable implant of the tumour, or to hasten the breakdown of the skin homografts.

Recently, Makari[5] introduced the Schultz–Dale technique for the detection of specific soluble antigen in the sera of carcinoma patients using uterine horns of female guinea pigs. Fink *et al.*[6] extended it to detect antibody production against tumours in mice.

Using the Schultz–Dale technique, we have made attempts to demonstrate the presence of antibody in the host during this passive transplantation tumour immunity. The criterion of immunization taken here is the ability of the host's uterus, sensitized in this way, to produce a contraction when exposed to the homologous tumour antigen *in vitro* in the Dale bath.

Pure line strains of Strong-*A* and *C*57 black mice were used and Crocker tumour was grafted into the subcutaneous tissues of both flanks. 6 draining axillary lymph nodes, 2 spleens, 1 kidney, ½ of a liver and 3 c.c. of serum were obtained from mice with 10–12-day-old growing Crocker tumours. These