SCREENING TESTS OF VARIOUS CHEMICAL SUBSTANCES AS CARCINOGENIC HAZARDS (Report 1)

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In 1956, I (1) reported that several dyes (rhodamine B, rhodamine 6 G, fluorescein sodium, eosine yellowish, acridine red (2) and toluleneblue (3)) were carcinogenic to experimental animals when repeatedly injected into the same site for a long period of time.

In the present paper are first given details of the similar injection experiments in which certain substances failed to produce sarcoma (Exps. I-IV; acid violet, auramine, sodium salicylate and 8-oxyquinoline sulfate). Feeding experiments with negative results of two substances (auramine G and salicylic acid) are next taken up (Exps. V and VI). These negative results are considered to be of some practical value since the substances tested are all food additives.

INJECTIONS OF ACID VIOLET, AURAMINE, SODIUM SALICYLATE AND 8-OXYQUINOLINE SULFATE

These substances are connected with the human life through their use as food additives in modern methods of food production and processing.

Experiment I. Acid violet was tested in this experiment. Acid violet (Colour Index No. 698; C₄₁H₄₄N₃O₆S₂Na) is 4-(4-(N-ethyl-p-sulfobenzylamino)-phenyl)-(4-(N-ethyl-p-sulfonium benzylamino)-pheny)-methylene)-N, N-dimethyl-2, 5-cyclohexadienimine (monosodium salt), forming violet powder, the watery solution of which has violet colour. The dye is used for coloring such food products as chocolate, candies, jellies, frozen desserts, beverages, icings, puddings, etc.

\[
\text{SO}_3\text{Na}
\]

The preparation of acid violet used in the experiment was a product of the
Tokushu Chemical Co., Ltd., Tokyo.

Experiment under the usual conditions was started with 16 normal rats of a mixed strain from the Saitama Prefecture, all weighing around 180 g.

Injections were made subcutaneously on the back of the rats at as nearly the same site as possible every time. The injection of 1 cc of 5.0 gdl distilled water solution of acid violet was repeated once every month, and after about 3 months, the dye solution was reduced to 0.5 cc. After about 9 months of repeated injections, the injections were discontinued.

8 rats survived 200 days or more, the longest survival period being 426 days (Table 1).

None of the animals developed tumor at the site of the injection. However, in the course of the experiment, one of them, dying on the 359th day, showed hypertrophy of thymus (2.0 x 1.4 x 1.2 cm), and another killed on the 426th day, had a cysticercus sarcoma of the liver. These tumors may be regarded as of the spontaneous origin, not connected with the experimental effect.

No significant change was noted in the local subcutaneous tissue as well as in internal organs at autopsy. The local tissues about the site of injection soon became deeply stained, and some violet color persisted for many months after the cessation of the injection.

**Experiment II.** Auramine (Colour Index No. 655; C_{17}H_{22}N_{3}Cl+H_{2}O) is hydrochloride of tetramethyldiamino-diphenyl-ketonimine, forming sulphur-yellow powder.

\[
\text{HCl} + \text{HN} = \text{C} \begin{array}{c}\text{N(CH}_3\text{)}_2 \\
\text{N(CH}_3\text{)}_2\end{array} + \text{H}_2\text{O}
\]

The preparation of auramine used in the experiment was the product of the Merck, Germany.

Animals used were 10 rats of hybrid strain (Saitama strain), all weighing around 150 g.

Auramine is insoluble in water and therefore it was suspended in water at the concentration of 0.5 gdl, and was injected subcutaneously on the back of the rats in 1 cc amounts, delivered into as nearly the same site as possible once or twice.
weekly as a rule, and after 20 days, the dye solution was increased to 2 cc. 5 rats survived 200 days or more, one of the highest longevity surviving 463 days (Table 2).

None of the animals developed tumor at the site of the injection. However, in the course of the experiment, one of them, dying on the 440th day, showed a mammary fibroadenoma (3.2×2.5×1.1 cm), presumably of the spontaneous origin.

Findings at autopsy: the local tissue at the site of the injections showed a fibrosis and hyaline degeneration. The liver was generally atrophic and histologically showed increase of Kupffer's stellate cells. Spleen showed some fibrosis.

Experiment III. Sodium salicylate (HOC₆H₄COONa) was tested in this experiment.

![COONa] [OH]

The preparation of sodium salicylate used in the experiment was the product of the Iwaki Seiyaku Co., Ltd., Tokyo.

Experiment was started with 25 normal adult albino rats of a mixed Saitama strain, all weighing around 150 g. The rats were given subcutaneous injections, at as nearly the same site as possible on the back, of 2 cc of 1.25 g/dl waterly solution of sodium salicylate once every week as a rule.

9 rats survived 300 days or more, one of the highest longevity surviving 550 days (Table 3).

None of the animals developed tumor at the site of the injection.

In the course of the experiment, two of the rats, dying on 294th and 391st days respectively, had a cysticercus sarcoma (spindle cell sarcoma) of the liver, and two others, dying on 460th and 495th days, showed hyper-

<table>
<thead>
<tr>
<th>Table 2. Experiment II.</th>
<th>Rat No.</th>
<th>Sex</th>
<th>Exp. days</th>
<th>Auramine Total (mg)</th>
<th>Inject. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>/</td>
<td>216</td>
<td>185</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>f</td>
<td>358</td>
<td>225</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>f</td>
<td>421</td>
<td>240</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>f</td>
<td>440</td>
<td>245</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>f</td>
<td>463</td>
<td>245</td>
<td>49</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3. Experiment III.</th>
<th>Rat No.</th>
<th>Sex</th>
<th>Exp. days</th>
<th>Sodium Salicylate Total (g)</th>
<th>Inject. No.</th>
<th>Final Body Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>m</td>
<td>305</td>
<td>0.825</td>
<td>32</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>2</td>
<td>m</td>
<td>370</td>
<td>1.0</td>
<td>40</td>
<td>220</td>
<td>195</td>
</tr>
<tr>
<td>3</td>
<td>m</td>
<td>378</td>
<td>1.025</td>
<td>41</td>
<td>195</td>
<td>/</td>
</tr>
<tr>
<td>4</td>
<td>m</td>
<td>391</td>
<td>1.0</td>
<td>39</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>5</td>
<td>m</td>
<td>395</td>
<td>1.0</td>
<td>39</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>6</td>
<td>m</td>
<td>460</td>
<td>1.1</td>
<td>43</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>7</td>
<td>m</td>
<td>495</td>
<td>1.225</td>
<td>48</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>8</td>
<td>m</td>
<td>500</td>
<td>1.5</td>
<td>59</td>
<td>210</td>
<td>/</td>
</tr>
<tr>
<td>9</td>
<td>m</td>
<td>550</td>
<td>1.4</td>
<td>55</td>
<td>240</td>
<td>/</td>
</tr>
</tbody>
</table>

59
trophies of the thymus (2.6×1.5×1.2 and 2.5×1.7×1.7 cm). Also one other, killed on 500th day, showed hypertrophy of the hypophysis (small pea size). All these tumors may be regarded as of the spontaneous origin.

The local tissue change was chiefly fibrosis. The liver was generally atrophic.

**INTRATESTICULAR INJECTIONS OF 8-OXYQUINOLINE SULFATE IN HAMSTERS**

**Experiment IV.** 8-Oxyquinoline sulfate which is used as a contraceptive agent was tested in this experiment, injecting it intratesticularly in hamsters.

\[
\text{\begin{tikzpicture}
    \node (n1) at (0,0) {OH};
    \node (n2) at (1,0) {N};
    \node (n3) at (2,0) {N};
    \draw (n1) -- (n2);
    \draw (n3) -- (n2);
    \draw (n2) -- +(0.5,0) node (s1) {H$_2$SO$_4$} -- +(0.5,0) -- cycle;
\end{tikzpicture}}
\]

The preparation of 8-oxyquinoline sulfate used in the experiment was the product of the Takeda Chemical Co., Ltd., Tokyo. Injections were made into the right testicle of the hamster. The injection of 0.05 cc of 10 gdl distilled water solution of 8-oxyquinoline sulfate, sterilized by heating before injection, was repeated once or twice weekly as a rule. Experiment was started with 12 normal hamsters, of which 5 survived 200 days or more, one of the highest longevity surviving 504 days (Table 4).

None of the animals developed tumor at the site of the injection.

The injected testicle was generally atrophic, and showed degeneration in the cases of Nos. 1 and 3. The liver was congested and showed hemosiderosis. Sometimes, in later stages, degeneration or necrosis of liver cells was noted. Spleen showed congestion, atrophy and hemosiderosis.

**Table 4. Experiment IV.**

<table>
<thead>
<tr>
<th>Hamster No.</th>
<th>Sex</th>
<th>Exp. days</th>
<th>8-oxyquinoline sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total (mg)</td>
</tr>
<tr>
<td>1</td>
<td>m</td>
<td>204</td>
<td>370</td>
</tr>
<tr>
<td>2</td>
<td>m</td>
<td>348</td>
<td>450</td>
</tr>
<tr>
<td>3</td>
<td>m</td>
<td>372</td>
<td>450</td>
</tr>
<tr>
<td>4</td>
<td>m</td>
<td>377</td>
<td>450</td>
</tr>
<tr>
<td>5</td>
<td>m</td>
<td>604</td>
<td>450</td>
</tr>
</tbody>
</table>

**AURAMINE G FEEDING IN MICE**

**Experiment V.** Auramine G (Colour Index No. 656; C$_{17}$H$_{29}$N$_3$Cl) is a hydrochloride of dimethyldiaminoditolyl-ketonimine, forming yellow powder. The substance is said to be used in Japan for coloring various food articles.

\[
\text{\begin{tikzpicture}
    \node (n1) at (0,0) {CH$_2$};
    \node (n2) at (1,0) {NH (CH$_3$)} -- (n1);
    \node (n3) at (2,0) {CH$_3$} -- (n2);
    \node (n4) at (1.5,-1) {NH (CH$_3$)} -- (n2);
    \draw (n3) -- +(-0.5,-0.5) -- cycle;
    \draw (n4) -- +(0.5,-0.5) -- +(-0.5,-0.5) -- cycle;
\end{tikzpicture}}
\]
The preparation of auramine G used in the experiment was the product of the Hodogaya Chemical Co., Ltd., Tokyo.

Experiment was started with 40 normal mice (both sexes) of a mixed Saitama strain, all weighing around 20 g. They were maintained on rice diet, to which auramine G was mixed evenly at the rate of 0.15%. The diet was supplemented with occasional supply of dried fish, cod-liver oil and green vegetables. The feeding of the dye was occasionally interrupted because of the toxic effect. 9 mice survived 150 days or more, one of the highest longevity surviving 369 days (Table 5).

None of the animals developed a tumor in any organ in the course of the experiment.

The liver showed degeneration of liver cells and increase of Kupffer's stellate cells. Spleen showed congestion, atrophy, fibrosis and increase of spleen cells.

### Table 5. Experiment V.

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Sex</th>
<th>Exp. days</th>
<th>Auramine G (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>m</td>
<td>156</td>
<td>228</td>
</tr>
<tr>
<td>2</td>
<td>m</td>
<td>156</td>
<td>228</td>
</tr>
<tr>
<td>3</td>
<td>f</td>
<td>159</td>
<td>234</td>
</tr>
<tr>
<td>4</td>
<td>m</td>
<td>180</td>
<td>288</td>
</tr>
<tr>
<td>5</td>
<td>m</td>
<td>260</td>
<td>474</td>
</tr>
<tr>
<td>6</td>
<td>f</td>
<td>316</td>
<td>598</td>
</tr>
<tr>
<td>7</td>
<td>m</td>
<td>352</td>
<td>679</td>
</tr>
<tr>
<td>8</td>
<td>f</td>
<td>359</td>
<td>694</td>
</tr>
<tr>
<td>9</td>
<td>m</td>
<td>369</td>
<td>718</td>
</tr>
</tbody>
</table>

Salicylic acid feeding in rats

The purpose of this experimental was to ascertain whether the feeding of salicylic acid, known to produce mutation, would cause pathological changes, especially malignant tumors, when continued for a long period of time.

**Experiment VI.** The preparation of salicylic acid used in the experiment was the product of the Koso Chemical Co., Ltd., Tokyo.

![Salicylic acid structure](https://via.placeholder.com/150)

Experiment was started with 34 normal rats of a mixed Saitama strain, all weighing around 200 g. They were maintained on rice diet, to which salicylic acid (C₆H₄(OH)CO₂H) was mixed evenly at the rats of 0.5-1.0 per cent. The diet was occasionally supplemented with dried fish, cod-liver oil, olive oil and green vegetables. The amount of salicylic acid added was 5 g per 1 kg of rice for about 8 months, increased to 10 g per 1 kg for the rest of the experimental period. The feeding of salicylic acid was continued without interruption.

9 rats survived 200 days or more, one of the highest longevity surviving 331 days (Table 6).
None of the animals developed tumor in any organ in the course of the experiment.

The stomachs of the experimental rats showed fibrosis and ulcer in a single case, dying on the 161st day (Total salicylic acid: 11.69 g). The liver showed cloudy hypertrophy. Spleen showed fibrosis, hemosiderosis and atrophy.

**DISCUSSION**

Miller and Pybus (4) reported that leukemia was produced by acid violet in the mouse. Yao (5) fed auramine mixed with the usual laboratory diet to rats, but he failed to find tumor. Carcinogenic activity of salicylic acid has been tested in the past by Hosino (6), but no indisputable malignant tumor was produced. In my experiments none of these substances showed any indication of carcinogenic action.

Attention may be called to the problems of coloring matters for various food articles and food additives as potential carcinogenic hazards which have sprung up in the world lately. Hueper (8), Truhaut (9), etc., have discussed these problems, and symposia on potential cancer hazards from chemical additives to food-stuffs took place in Bad Godesberg in 1954 (7) and again in Rome (1956) under the sponsorship of the International Union against Cancer.

In view of these facts, the negative data presented in this paper would seem to be of sufficient interest to be reported.

**SUMMARY**

1. Under the conditions of the above described experiments, acid violet, auramine, sodium salicylate, 8-oxyquinoline sulfate, auramine G and salicylic acid did not show carcinogenic activity.

2. No marked change in internal organs was found in the rats, hamsters and mice under the conditions of the respective experiments. It may be noted,
however, that auramine produced some degeneration of liver cells in mice and salicylic acid a mild atrophy of spleen in rats.

ACKNOWLEDGEMENTS

I take pleasure in acknowledging my indebtedness to Dr. Waro Nakahara and Dr. Makoto Tanaka for their help and encouragement in the course of this work.

REFERENCES

(7) Summary of a meeting of West European Scientists on the prophylaxis of cancer. 1954.
要 旨

食品添加用諸化学物質の発癌実験（第1報）

梅 田 真 男（癌研究所）

1956年にヨーマ発癌の国際会議が開催され、食品、石鹸、化粧品の製造や保存に用いる色素や添加物の発癌性についても論述され、この問題が各国において重要視されている現在、私は下記の6種類の食品添加物について、発癌性の問題を研究した。

<table>
<thead>
<tr>
<th>物 質 名</th>
<th>投 与 法</th>
<th>実験 動 物</th>
<th>最高生存日数</th>
<th>癌発生数</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Violet</td>
<td>皮下注射</td>
<td>ダイクロネズミ</td>
<td>426日</td>
<td>0</td>
</tr>
<tr>
<td>Auramine</td>
<td>皮下注射</td>
<td>ダイクロネズミ</td>
<td>463日</td>
<td>0</td>
</tr>
<tr>
<td>Sodium Salicylate</td>
<td>皮下注射</td>
<td>ダイクロネズミ</td>
<td>550日</td>
<td>0</td>
</tr>
<tr>
<td>8-Oxyquinoline Sulfate</td>
<td>鼻腔注射</td>
<td>ハムスター</td>
<td>504日</td>
<td>0</td>
</tr>
<tr>
<td>Auramine G</td>
<td>経口</td>
<td>ハツカネズミ</td>
<td>369日</td>
<td>0</td>
</tr>
<tr>
<td>Salicylic Acid</td>
<td>経口</td>
<td>ダイクロネズミ</td>
<td>331日</td>
<td>0</td>
</tr>
</tbody>
</table>

以上の表の如く、アンリドバイオレット、オーラミン、サリチル酸ソーダ、8-オキシキノリン硫酸塩の注射実験、及びオーラミンG、サリチル酸の経口投与実験では、動物に癌はできなかった。しかしオーラミンGは肝臓に変性を、またサリチル酸は肺の萎縮著を起した。現在種々の食品添加物について研究中である。これらの研究が癌と食生活の問題への寄与となれば幸と思う。

（厚生科学研究費による）