The mechanism of vitamin A induced teratogenesis

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WITH TWO PLATES

It is generally recognized that some congenital malformations may be caused by an abnormal environment surrounding the mother in pregnancy. Many experiments have been reported concerning the teratogenesis due to various hypovitaminoses, but there are relatively few reports on teratogenesis resulting from the administration of a large quantity of vitamin. Among the latter, the best known are malformations caused by hypervitaminoses A and D.

In spite of many experiments on the effect of hypervitaminosis A carried out by Cohlan (1953, 1954, 1961), Giroud & Martinet (1955, 1956, 1957, 1959, 1960), Millen & Woollam (1957a, 1957b, 1958a, 1958b, 1958c, 1961), Kalter & Warkany (1961), Här tel & Här tel (1960), and also by many Japanese workers (Inaba, 1958; Nakamura, 1958; Araki, 1958; Yukioka, 1958; Tsuruhara, 1959; Kuzukawa, 1960), the mechanism of such a teratogenesis still remains obscure. Cohlan (1954) and Giroud (1957) considered a direct action of vitamin A on the foetus, because vitamin A content in the liver of the foetus was markedly increased when the former was given in excessive dose to the mother in pregnancy. Takekoshi (1961a, 1961b) observed that cortisone, trypan blue, urethane and chondroitin sulfate enhanced the teratogenic activity of vitamin A, and that the adrenal weight of animals given a large dose of vitamin A was increased, while the lipid droplets of the adrenal cortex became minute in size and increased in number. From these facts, he concluded that excessive production of adrenal cortical hormone in these cases might not be negligible as compared to the direct action of vitamin A on the foetus.

It may be easily assumed that the intimate reciprocal actions between various hormones and vitamins are necessary for the normal development of foetuses. Therefore, in the present study, special attention was paid to thyroid function in animals which were given a large amount of vitamin A, and the relationship

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between blood concentration of thyroid hormone and teratogenesis due to vitamin A was investigated.

**MATERIAL AND METHODS**

**Experiments on teratogenesis**

Female mice of ddN strain weighing about 25 g. were used. The day on which a copulation plug was observed was taken as day 0 of pregnancy. The mice were divided into four groups, and the following treatments were performed daily for 4 days from the 10th to the 13th day of pregnancy.

Group 1, intramuscular injection of 0.2 ml. (30 mg.) of Tween 80 (the vitamin A solvent); group 2, intramuscular injection of 10,000 I. U. of vitamin A; group 3, intraperitoneal injection of 8 mg. of methylthiouracil (MTU); and group 4, intramuscular injection of 10,000 I. U. of vitamin A plus intraperitoneal injection of 8 mg. of MTU.

On the 17th day of pregnancy, animals of all these groups were sacrificed under ether anesthesia, the foetuses were taken out and examined macroscopically for the existence of malformations. Skeletons of the foetuses were studied by the method of Dawson (1926). (The vitamin A was a product of Eisai Co., Tokyo, and the MTU was a product of Chugai Pharmaceutical Co., Tokyo.)

**Effects of vitamin A on thyroid function and on adrenal and testicular weights**

It is well known that some changes in thyroid function may be elicited by pregnancy and by estrogen administration. Because the thyroid of the mouse is too small to be examined for its detailed function and it is impossible to obtain enough blood from one mouse in order to enable determination of protein bound iodine (PBI), male rats of Wistar strain were used. They were maintained on low-iodine food for 10 days prior to sacrifice in order to elevate $\text{I}^{131}$ uptake.

Twenty-seven rats were divided into four groups, and subjected to the following treatments daily for 4 days: Group 1, intramuscular injection of 150 mg. (1 ml.) of Tween 80; group 2, intramuscular injection of 50,000 I. U. (1 ml.) of vitamin A; group 3, intraperitoneal injection of 20 mg. of MTU; and group 4, intramuscular injection of 50,000 I. U. of vitamin A plus intraperitoneal injection of 20 mg. of MTU.

The animals of all groups were given intraperitoneal injection of 1 $\mu$g. of $\text{I}^{131}$, 24 hr. before being sacrificed. At the time of sacrifice, thyroid, adrenal and testis were taken out, weighed by a torsion balance, and the weight of each organ per 100 g. body weight was computed. The 24-hr. $\text{I}^{131}$ uptake by thyroid was determined with a well-type scintillation counter. For the determination of serum PBI, blood was taken from the jugular vein at the time of sacrifice.
Fig. A. A frontal section through the face of a mouse foetus with the cleft palate induced by excess vitamin A. Hyperplasia of the cartilage of the nasal septum. C: Abnormal hyperplasia of the septal cartilage; N: Nasal septum; P: Palate; T: Tongue. ×36.

Fig. B. The foreleg of normal mouse foetus. ×5.

Figs. C, D. Various types of finger defect found in the group given both MTU and vitamin A. ×5.

Fig. E. The hind leg of normal mouse foetus. ×5.

Figs. F, G, H, I. Various types of toe defect found in the group given both MTU and vitamin A. ×5.

S. TAKEKOSHI
FIG. J. A 17-day normal mouse foetus. $\times 2.5$.

FIG. K. A 17-day abnormal mouse foetus found in the group given both MTU and vitamin A. Note the peculiar shape of limbs. $\times 2.7$.

FIG. L. Skeleton of a 17-day normal mouse foetus. $\times 3$.

FIG. M. Skeleton of a 17-day abnormal mouse foetus. Reduction in long-bone formation. $\times 3$.

S. TAKEKOSHI

(Facing page 265)
Vitamin A induced teratogenesis

RESULTS

Teratogenic effects of vitamin A

As shown in Table 1, no malformation was induced in the Tween 80 group. From the group which was given 10,000 I.U. of vitamin A, 34 per cent. of the foetuses showed cleft palate, and 71.2 per cent. had some kind of macroscopically observable malformation. On the other hand, no malformations were observed in the group given MTU alone, whereas in the group given both MTU and vitamin A, the number of abnormal animals was increased. Cleft palate and digital malformations were observed in all the foetuses of this group.

The palate of the foetus often showed a complete cleft palate, and the nasal septa were widened due to the abnormal hyperplasia of the cartilage (Plate 1, Fig. A).

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Number of dams</th>
<th>Number of living embryos</th>
<th>Number of young with cleft palate (%)</th>
<th>Number of young with finger malformation (%)</th>
<th>Number of young with toe malformation (%)</th>
<th>Number of young with any kind of malformation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tween 80</td>
<td>12</td>
<td>104</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Vitamin A 10,000 I.U.</td>
<td>11</td>
<td>94</td>
<td>(34.0%)</td>
<td>14</td>
<td>65</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>MTU 8 mg.</td>
<td>10</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Vitamin A 10,000 I.U. and MTU 8 mg.</td>
<td>12</td>
<td>60</td>
<td>(100%)</td>
<td>(90.2%)</td>
<td>(100%)</td>
<td>(100%)</td>
</tr>
</tbody>
</table>

Various types of limb defects, namely oligodactyly or syndactyly in both the fore- and hind-legs are observed (Plate 1, Figs. B, C, D, E, F, G, H, I). Furthermore staining of the skeleton revealed that the limb defects were caused by the reduction, or in the worst cases the complete absence, of long bones such as the humerus, radius, ulna, femur, tibia or fibula (Plate 2, Figs. L, M). The appearance of such deformed foetuses may closely resemble the phocomelia (Plate 2, Figs. J, K) attributed to thalidomide taken during pregnancy.

Effects of vitamin A on thyroid weight, thyroidal I\(^{131}\) uptake, serum PBI, adrenal and testicular weights

As shown in Table 2, the thyroid weight evidently increased in vitamin A given animals, and at the same time thyroidal I\(^{131}\) uptake was elevated. Serum
PBI was determined on the animals given an excess of vitamin A, and was found remarkably decreased. With group 4, which received combined administration of vitamin A and MTU, more remarkable goitres were produced than in group 3, which was given MTU alone.

**TABLE 2**

*Effect of vitamin A on thyroid weight, thyroid $I^{131}$ uptake, serum PBI, adrenal and testicular weight*

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Body weight (g.)</th>
<th>Number of animals</th>
<th>Thyroid weight (mg.)</th>
<th>Thyroidal $I^{131}$ uptake (y/dl.)</th>
<th>Serum PBI (mg.)</th>
<th>Adrenal weight (mg.)</th>
<th>Testis weight (g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tween 80 150 mg.</td>
<td>107±3</td>
<td>7</td>
<td>13.1±0.8</td>
<td>38.4±3.9</td>
<td>3.7±0.3</td>
<td>25.5±0.9</td>
<td>1.49±0.11</td>
</tr>
<tr>
<td>2</td>
<td>Vitamin A 50,000 I.U.</td>
<td>90±3</td>
<td>7</td>
<td>17.1±1.6</td>
<td>50.2±3.0</td>
<td>2.4±0.3</td>
<td>29.6±3.2</td>
<td>1.42±0.10</td>
</tr>
<tr>
<td>3</td>
<td>MTU 20 mg.</td>
<td>110±4</td>
<td>7</td>
<td>20.1±0.9</td>
<td>6.9±1.2</td>
<td>2.7±0.2</td>
<td>23.7±1.8</td>
<td>1.24±0.11</td>
</tr>
<tr>
<td>4</td>
<td>Vitamin A 50,000 I.U. and MTU 20 mg.</td>
<td>95±5</td>
<td>6</td>
<td>25.8±2.2</td>
<td>11.8±2.0</td>
<td>2.1±0.2</td>
<td>44.7±3.9</td>
<td>1.41±0.16</td>
</tr>
</tbody>
</table>

Results expressed as mean ± standard error of mean.

Administration of vitamin A did not elicit any change in testicular weight, but apparently increased adrenal weight. A similar result was earlier obtained with mice (Takekoshi, 1961a). Further, it is important to note that more increase in adrenal weight was obtained by combined administration of vitamin A and MTU than by administration of vitamin A alone.

**DISCUSSION**

According to an article by Drill (1943), McCarrisson discovered as early as 1923 that cod-liver oil retarded metamorphosis of tadpoles, and in 1936 Rokhlina observed that carotene had the same effect on axolotls, and in 1933 and 1935 Eufinger and Gottlieb found that when vitamin A was administered to tadpoles, it depressed the metamorphosis-accelerating action of thyroxin. On the other hand there are reports that the administration of vitamin A decreased the size of the thyroid gland, that it lowered oxygen consumption (Logaras & Drummond, 1938; Sadhu, 1948; Sadhu & Brosy, 1947), and that it diminished serum PBI (Donowski et al., 1955). Sometimes vitamin A was clinically used for treatment of hyperthyroidism (Dietrich, 1936). All these reports indicate that a large dose of vitamin A can elicit some changes in thyroidal function.

It was found in our experiments that the blood concentration of thyroid hormone was significantly reduced in vitamin A treated animals, while thyroid
weight and thyroidal uptake of $^{131}$I were significantly increased. It can therefore be considered that the blood concentration of thyroid hormone which was lowered by MTU would have further been lowered by the addition of vitamin A, consequently producing more significant goitre in group 4. The result was just as anticipated from the viewpoint of the feedback theory (Hoskins, 1949). But why was the blood concentration of thyroid hormone lowered by the administration of vitamin A? This point has already been discussed in detail by Shichijo & Shimoda (1962), and it was shown that vitamin A accelerates de-iodination of thyroxin and the concentration of circulating thyroid hormone may be decreased as a result.

On the other hand there are some reports that hypothyroidism of the mother may cause the congenital malformation of her child (Hodge et al., 1952; Eliphinstone, 1953), and that congenital malformations occur frequently in such areas where endemic goitre is prevalent (Pasma, 1948). Langman et al. (1955) succeeded in producing malformations of the eye, cleft palate and harelip in the young by performing partial thyroidectomy or by giving MTU to the mother animal to induce hypothyroidism.

From these facts it is concluded that a fall in the blood concentration of thyroid hormone may have a close connection with teratogenesis due to vitamin A. If this is right, the administration of vitamin A in combination with antithyroidal substance must produce more malformations. Already Millen & Woollam (1958a) reported that when rats were orally given both vitamin A (from the 9th to 13th day of pregnancy) and MTU (from the 1st to the 9th day of pregnancy), teratogenesis was significantly greater than when vitamin A alone was given. Also, in the present experiment in which mice received injections of vitamin A and MTU from the 10th to 14th day of gestation, the results were exactly the same as those of Millen & Woollam, namely that the teratogenic action of vitamin A was enhanced by the simultaneous administration of MTU. The result can be explained by assuming that combined administration of vitamin A and MTU reduces the blood concentration of thyroid hormone more than the administration of MTU alone.

It has already been reported that trypan blue and vitamin A might also act synergistically in teratogenesis (Takekoshi, 1961b) (Table 3). Since Yamada (1960a, 1960b) observed that the administration of trypan blue decreased thyroid weight, thyroidal uptake of $^{131}$I and blood concentration of PBI, and Shimoda et al. (1962) assumed that trypan blue might probably exert inhibitory action on TSH secretion from the hypophysis, a similar explanation may be applied to the teratogenic activity of the combination of trypan blue and vitamin A. The combined administration of these two, both of which reduce PBI, may enhance the rate of producing malformations.

From the facts described above, it is evident that the mechanism of teratogenic action of vitamin A may involve the thyroid gland as well as the adrenal gland, on which the author reported previously (Takekoshi, 1961a). Teratogenic
Table 3

Combinative teratogenic effect of vitamin A and trypan blue on mice embryos

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of dams</th>
<th>Number of living embryos</th>
<th>Number of young with cleft palate (%)</th>
<th>Number of young with tail malformation (%)</th>
<th>Number of young with any kind of malformation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% trypan blue 0-2 c.c.</td>
<td>10</td>
<td>59</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Vitamin A 5,000 I.U.</td>
<td>11</td>
<td>97</td>
<td>0</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>1% trypan blue 0-2 c.c. and vitamin A 5,000 I.U.</td>
<td>15</td>
<td>58</td>
<td>6</td>
<td>5</td>
<td>12</td>
</tr>
</tbody>
</table>

1 per cent. trypan blue solution was injected subcutaneously on the 7th day of pregnancy. Vitamin A was injected intramuscularly for 4 days from the 10th to 13th day of pregnancy.

action of vitamin A, however, can by no means be explained by dysfunction of these endocrine glands only, for if malformation were simply the result of the fall of blood concentration of thyroid hormone, the administration of MTU alone would produce frequent malformations. But under the condition of the present experiments—with regard to the time and duration of MTU administration—no teratogenesis could be induced with MTU alone. It may, of course, be considered that the total dose of MTU in the present experiments was insufficient to lower the blood concentration of thyroid hormone, but at the same time one cannot deny the possibility that some unknown factor may be involved in the mechanism of teratogenesis besides endocrine dysfunction.

For these reasons it is impossible to draw any definite conclusion as to the mechanism of teratogenic action of vitamin A at the present time. But at least the present experiments have shown that there is a close relation between teratogenesis and fall of the blood concentration of the thyroid hormone.

**SUMMARY**

Investigations were carried out with ddN strain mice on the teratogenic effects of large doses of vitamin A. At the same time the relationship between the teratogenesis and thyroid function was examined with Wistar rats in order to elucidate the mechanism of the teratogenesis. The following results were obtained.

1. Administration of 10,000 I.U. of vitamin A alone to pregnant mice produced malformations in 71.2 per cent. of their living foetuses. Administration of 8 mg. of MTU alone could not produce any malformations, whereas combined administration of 10,000 I.U. of vitamin A and 8 mg. of MTU produced malformations in 100 per cent. of the foetuses.

2. Administration of 50,000 I.U. of vitamin A elicited a remarkable increase
in thyroid weight and in thyroidal uptake of I$^{131}$, and a remarkable fall in blood concentration of thyroid hormone.

3. Combined administration of 20 mg. of MTU and 50,000 I.U. of vitamin A produced more remarkable goitre than did the administration of 20 mg. of MTU alone. For this reason it is considered that the blood concentration of thyroid hormone, which is lowered by the administration of MTU, might have dropped still more by the addition of vitamin A.

4. In vitamin A treated animals, there were no changes in testis weight, but evident increase in adrenal weight. This tendency was still more significant when MTU was also given.

5. From these data it was suggested that besides the adrenal gland, the thyroid gland has a close connection with teratogenesis due to vitamin A. A fall in blood concentration of thyroid hormone was especially emphasized as one of the important factors.

RÉSUMÉ

Le mécanisme de la tératogénie induite par la vitamine A

Les recherches ont été effectuées sur la souche de Souris ddN pour éprouver les effets tératogéniques de hautes doses de vitamine A. En même temps, on a étudié la relation entre la tératogénèse et la fonction thyroidienne sur des rats Wistar, afin d'élucider les mécanismes tératogéniques.

1. L'administration de 10,000 U.I. de vitamine A seule à des souris gestantes a provoqué des malformations chez 71,2% des foetus vivants. L'administration de 8 mg. de MTU seul ne provoque aucune malformation, tandis que l'administration combinée de 10,000 U.I. de vitamine A et de 8 mg. de MTU provoque des malformations chez 100% des foetus.

2. L'administration de 50,000 U.I. de vitamine A provoque une importante augmentation du poids de la thyroïde, de l'assimilation thyroidienne de I$^{131}$, et une chute importante de la concentration de l'hormone thyroidienne dans le sang.

3. L'administration combinée de 20 mg. de MTU et de 50,000 U.I. de vitamine A pour effet de provoquer des goitres plus importants que l'administration de MTU seul. On peut donc admettre que la concentration de l'hormone thyroidienne dans le sang, qui est diminuée par l'administration de MTU, peut s'abaisser plus encore par l'addition de vitamine A.

4. Chez les animaux traités par la vitamine A, il n'y a aucun changement de poids des testicules, mais une augmentation manifeste du poids des surrenales. Cette tendance est encore plus significative, quand on donne en plus du MTU.

5. Ces données suggèrent qu'à côté de la surrenale, la thyroïde est en liaison étroite avec les malformations provoquées par la vitamine A. Une chute de la concentration d'hormone thyroidienne dans le sang peut être considérée comme un facteur important dans le mécanisme tératogénique.
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REFERENCES


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