Developmental Anomalies in Offspring of Pregnant Mice Treated with Nicotine

It has been known for some time that nicotine is among other things a so-called mitotic poison. Brock *et al.* (*1*) reported that this chemical severely impedes the mitosis of fertilized ovum of the sea urchin, and according to Mainx (*2*) it induces an abnormal mitosis in the epithelium of the oral cavity of the salamander. Although, from a hygienic point of view, the effects of chronic nicotine poisoning on reproduction in the Muridae and on the vitality of the offspring have been studied (*3*), yet no investigation of the effects of nicotine on the development of the offspring has been made. This led us to undertake a detailed investigation of the effects of this chemical on the development of embryos by its transitional application to pregnant mice.

As material, mice of the S strain obtained from the Japanese National Institute of Genetics were used. Female mice 2 to 5 months old were mated, and 230 mice which had been diagnosed as pregnant were used. Between 5 and 15 days after mating, nicotine (0.1 percent aqueous solution) prepared by Merck and Co., Ltd., was injected subcutaneously or intraperitoneally once, or repeated on two or three consecutive days. Most of the animals were sacrificed at term, but some were opened in the period of mid-pregnancy. Then the state of pregnancy and the development of the offspring were investigated; the results are listed in Tables 1 and 2.

As controls, 225 full-term fetuses removed from 29 untreated mice were used; only five cases of resorption were found, and there were no cases with malformations.

Tables 1 and 2 indicate that nicotine, when it is administered during certain periods of pregnancy, has a remarkable lethal effect. In many litters, all the embryos seemed to have died immediately after injection. Sometimes, however, we saw embryos which had developed for a few days after injection and had then died. The litter size of the nicotine-treated mice, whose pregnancy did not undergo complete resorption, was significantly different from that of the controls. Although weights are not listed in the tables, we should note that the average body weights of the surviving embryos were slightly less than those of the control group, but no significant difference was recognized.

Next, it was confirmed that nicotine often showed conspicuous teratogenic effects when it was administered during the mid-term of pregnancy. The effect of three injections given between the 9th and 11th days was more severe than that of one injection given on any other day. Malformations, in almost all cases, belonged to the skeletal system. Defects in the limbs were especially predominant in number. Among them, digits with maldirection were most frequent. Besides the occurrence of the previously mentioned defects, an appreciable number of malformed ankle, elbow, and wrist joints, spinal curvature, and a few cleft palates were observed. It was also shown that the later in gestation the mice were opened, the more anomalies of the limbs were to be observed in the embryos. The critical period for the digital anomalies was assumed to be the 6th to the 14th day. It is to be noted that the highest frequency was recognized in the period from the 7th to the 12th day.

Histologically it was recognized that in the case of a digit with maldirection, its joints were malformed, or bones were deviated. In the case of pes varus or pes valgus, there was an enlargement of the articular cavity in the tibiotarsal joint or an irregularity of the metatarsal bones. In the case of malformed elbow joints, the trochlea humeri was malformed. In the case of spinal curvature, wedge-shaped vertebral bodies were recognized. Moreover, the over-all osteogenesis was retarded in the bones of the respective joints.

It was also found that in macroscopic or histological observations, placentae of the malformed embryos were normal except in one case in which a fairly large hemorrhagic area was recognized.

Now it must be pointed out that the administration of the chemical before the ninth day of gestation, when the

Table 1. Effects of nicotine (in 0.1 percent aqueous solution) injection on pregnant mice observed at term.

Day of injection	Dosage per day (mg/g) and method of injection*	Total No. of mice	No. of pregnancies undergoing complete resorption	Av. litter size	Fetuses			Type and No. of anomalies†
					Total No.	Dead (%)	Congenital anomalies (%)	
5	0.025 (s.c.)	6	3	4.3	13	0	0	
6	0.025 (s.c.)	17	6	8.1	89	15	9	4T, 3D(t), 1P
7	0.025 (s.c.)	15	5	8.1	81	14	26	2Cu, 7D(f), 8P, 12D(t), 2T
8	0.025 (s.c.)	15	5	7.0	70	17	21	1Cu, 1M, 5P, 10D(t), 3D(f), 1T
9, 10, 11	0.025 (s.c.)	44	23	8.0	168	11	64	9Cu, 4M, 39D(f), 3Sd(f), 1Bd(f), 89D(t), 3Sd(t), 1Pd(t), 36T, 12P
9, 10, 11	0.008 (i.p.)	34	13	7.5	158	15	40	6Cu, 3M, 22D(f), 2Sd(f), 7P, 55D(t), 1Sd(t), 3T
12	0.025 (s.c.)	13	3	6.5	65	32	18	1Cu, 1M, 4D(f), 5P, 5D(t)
13	0.025 (s.c.)	14	3	7.1	78	2	10	4P, 4D(t), 1Ad(t), 1Bd(t)
14	0.025 (s.c.)	10	1	7.2	65	11	11	3Cu, 2D(f), 2P, 3D(t), 2Pd(t)
15	0.025 (s.c.)	14	1	7.4	96	5	0	

* Abbreviations: s.c., subcutaneous; i.p., intraperitoneal.
† Anomalies: Ad(t), adactyly (toe); Bd(f), brachydactyly (finger); Bd(t), brachydactyly (toe); Cu, malformation of elbow joint; D(f), malformation of joint of finger; D(t), malformation of joint of toe; M, malformation in wrist joint; P, malformation in ankle joint (mostly pes varus, occasionally pes valgus); Pd(t), polydactyly (toe); Sd(f), syndactyly (finger); Sd(t), syndactyly (toe); T, spinal curvature.

Table 2. Effects of subcutaneous injection of nicotine (0.1 percent aqueous solution) on pregnant mice observed in the period of mid-pregnancy.

Day of injection	Dosage per day (mg/g)	Total No. of mice	Day sacrificed	Av. litter size	Fetuses			Type and No. of anomalies*
					Total No.	Dead (%)	Congenital anomalies (%)	
8, 9	0.025	6	14	7.3	44	11	2	1T
8, 9	0.025	10	15	6.2	62	5	35	8D(f), 15D(t), 2T, 1He
8, 9	0.025	10	16	8.0	80	6	35	4P, 3D(f), 22D(t), 3Sd(t), 1Sd(f), 3T, 2Bd(t)
8, 9	0.025	7	17	6.7	47	21	41	13D(t), 1Ad(f), 2Bd(f), 1Sd(f), 4T, 1Ps, 1Ba, 1Pa, 1Hu

* Anomalies: Ad(f), adactyly (finger); Ba, shortness of antebrachium; Bd(f), brachydactyly (finger); Bd(t), brachydactyly (toe); D(f), malformation of joint of finger; D(t), malformation of joint of toe; He, hematoma in head region; Hu, umbilical hernia; P, malformation in ankle joint (mostly pes varus, occasionally pes valgus); Pa, eyelids open; Ps, cleft palate; Sd(f), syndactyly (finger); Sd(t), syndactyly (toe); T, spinal curvature.

primordium of the limbs appears, also induces anomalies. For this fact, we are at present not able to give a definite conclusion—that is, the possibility that this chemical affects the cells of the earlier stage than the primordium or that its action continues in the embryo for many days could not be decided. There are many chemical agents, which induce teratogenic effects mainly on the skeletal system, such as nitrogen mustard (*4*), ethylurethan (*5*), 8-azaguanine (*6*), 2,3-dimercaptopropanol (BAL) (*7*). But it is peculiar to the case of nicotine treatment that the critical period for anomalies in limbs is so long and that malformations so often occur in the various joints.

It may be concluded that we confirmed that nicotine has a lethal effect upon embryos of mice and also has a powerful teratogenic effect on their skeletal system when it is administered during pregnancy.

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25 November 1957

Effects of 8-Methoxypsoralen and Ultraviolet Light in Human Skin

Extracts of *Ammi majus* (Linn.) have been used by the Egyptians as pigmenting agents for centuries (*1*). Recently one of the active ingredients of this plant (8-methoxypsoralen) has been publicized by magazines and newspapers as the "sun-tan pill." This substance has been used in the treatment of vitiligo, a disease of human skin in which circumscribed spots of the skin stop forming pigment. 8-Methoxypsoralen has been shown to be a photosensitizer (*2*) but, paradoxically, many individuals have reported that the ingestion of this substance protects them against sunburn (*3*).

The following experiment (*4*) was performed to clarify the mechanism by which 8-methoxypsoralen and sunlight alter the physiological reactions of human skin. The subject (blond, blue-eyed, healthy, 33-year-old white male) exposed an area of the left thigh to ultraviolet irradiation with a mercury vapor lamp at 30 inches distance and with exposure times of 1, 3, 5, 5, 5, 5, 10, 10, 15, 15, 15, 15, 15, and 15 minutes on successive days. Afterward a similar area of the right thigh was exposed in the same manner, but in this second series of irradiations, 20 mg of 8-methoxypsoralen was taken by mouth 2 hours before each exposure. The ultraviolet irradiation was of low intensity, and no clinical redness was produced at any time. Specimens of skin for microscopic examination were removed from the irradiated areas of both thighs on the 7th and 14th days of the irradiation series.

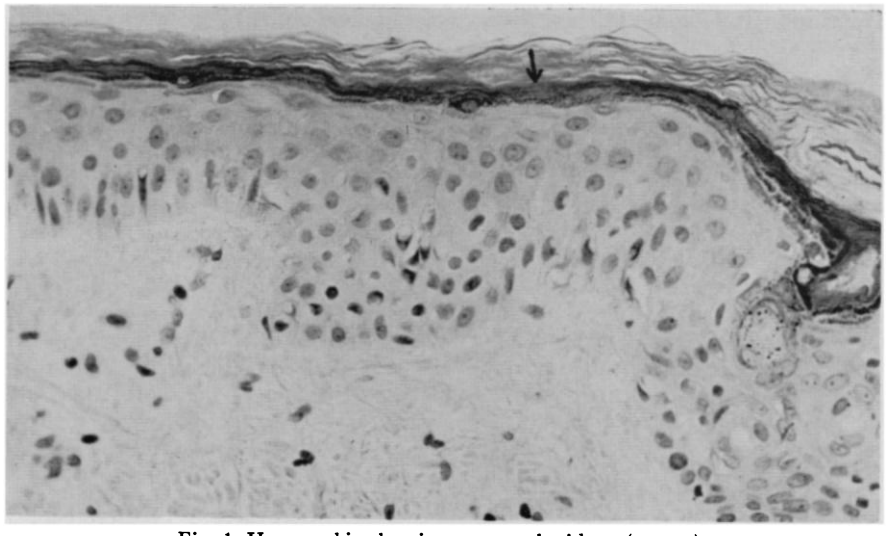

Fig. 1. Human skin showing stratum lucidum (arrow).

After 14 days of irradiation, the threshold erythema dose of ultraviolet light in the treated area of the left thigh was 25 minutes; 50 minutes of irradiation to the corresponding area of the right thigh produced no redness. Microscopic examination of the specimens of skin revealed that the most prominent change occurred in the horny layer. The horny layer had thickened in both treated areas, but the specimen from the right thigh also revealed the formation of a stratum lucidum. Normally, a stratum lucidum is seen only in areas where the epidermis develops a thick horny layer —for example, on palms and soles. Physiologically, it is never seen in the skin of the thigh. The thickening of the stratum corneum and formation of a stratum lucidum appears to represent the initial reaction of the skin in response to 8-methoxypsoralen and sunlight. There was no melanin present in the stratum corneum in these specimens, so the increased tolerance to ultraviolet light was due solely to the alteration in the horny layer. Miescher (*5*) has shown that ultraviolet irradiation produces thickening of the horny layer; such thickening constitutes the primary protective mechanism of the skin against ultraviolet irradiation. Microscopic examination of skin from the back of an individual who had been using 8-methoxypsoralen and sunlight for 2 months revealed a prominent stratum lucidum (Fig. 1.).

The combination of ultraviolet light and 8-methoxypsoralen resulted in more new pigment formation in the treated area of the right thigh than in the corresponding control area of the left thigh. The quantity of newly formed pigment was not great. Examination of the skin from the individual who had used 8-methoxypsoralen for 2 months revealed an abnormally large amount of pigment in both the basal cell layer and the horny layer. The pigment-forming cells and their dendritic processes were not filled with melanin. The absence of pigment in the dendritic processes would indicate that no great amount of pigment was being formed at the time the specimen of skin was removed. It appears from these findings that, while 8-methoxypsoralen increases the pigmenting effect of ultraviolet light, the development of the psoralen tan is due primarily to retention of pigment in the skin. The psoralen tan has a curious quality (*6*), and this may be due to the large amount of melanin retained in the altered horny layer.

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29 November 1957