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Congenital Malformations in Offspring of Mice Treated with Caffeine.* (25757)

HIDEO NISHIMURA AND KAZUO NAKAI (Introduced by M. M. Nelson)

Dept. of Anatomy, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan

Certain substances which act as mitotic poisons have been reported to have teratogenic effects in mammalian embryos(1,2). However, many chemicals present in foods, beverages or drugs have not been tested or have been insufficiently studied for possible teratogenic effects. A few studies on caffeine have been reported. Druckrey and Schreiber(3) observed cessation or irregularity in mitosis in fertilized eggs of sea urchin after these were dipped in a caffeine solution. Eichler and Mügge(4) found no deleterious effects on embryonic development when pregnant rats were injected subcutaneously with large doses of caffeine. However, continuous treatment of pregnant rabbits with high doses of caffeine resulted in occasional early embryonic death or retarded development; the newborn young appeared to have "poor resistance"(5-7). Since no congenital malformations have been reported, we have investigated the effects of high doses of caffeine injected in pregnant mice.

Methods. Approximately 100 pregnant mice of the SMA strain (originating from Japanese Nat. Inst. of Genetics) were given a single intraperitoneal injection of 1% caffeine once during 7th to 15th days of pregnancy. A dosage level of 0.25 mg/g body weight, considered to be approximately the maximum dose tolerated, was used. The animals were sacrificed near term or in mid-pregnancy and fetuses weighed, examined for anomalies, and fixed in 20% formalin solution. Fetuses obtained from 25 normal pregnant mice were used as controls.

Results. Table I shows that caffeine administration may result in embryonic death or in malformed fetuses. Incidence of embryonic death was highest for injections given during 7th-12th days, when resorption of all embryos of a litter was sometimes induced. However, any injection given between 7th-14th days resulted in some dead or macerated fetuses. Malformations were observed in 18 to 43% of fetuses when injections were given between 10th and 14th days; a single malformation was found for injections on 9th day

TABLE I. Effects of Intraperitoneal Injection of Caffeine* in Pregnant Mice Observed at Term.

Day of inj.	No. of mice	No. of complete resorptions	Young		
			Total No.	% dead	% abnormal
7	4	2	11	18	0
8	5	0	36	6	0
9	11	2	45	11	2
10	6	0	40	3	38
11	10	0	57	12	19
12	8	1	41	17	41
13	7	0	51	8	43
14	7	1	44	18	18
15	3	0	24	0	0
Uninjected controls	25	0	225	2	0

* 1% aqueous solution, 0.25 mg/g body wt.

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TABLE II. Number and Types of Malformations Observed in Offspring of Pregnant Mice Given Intraperitoneal Injections of Caffeine.*

Day of inj.	Abnormal young No.	No. of young with specific malformations in: Digits Fore	Digits Hind	Joints†	Palate	Hematoma	Other
9	1				1		
10	15	3	10	3	2		1
11	11	8	8		1	1	
12	17	14	9	4	10	12	
13	22	14	7	2	9	15	1
14	8	1	8	2		4	

* 1% aqueous solution, 0.25 mg/g body wt.
† This column includes clubfoot as well as joint malformations.

of pregnancy. No significant effects on embryonic body weights were observed. All placentae were normal in size, shape, and color and histological study of representative placentae for each litter did not reveal any morphological changes.

The malformations observed were predominantly those of skeletal system, especially digital defects and cleft palate (Table II). Digital defects included angulation, brachydactylism, syndactylism, adactylism, and polydactylism. The critical period for adactylism in forefeet was 10th-13th days and in hindfeet 11th-14th days. Clubfoot (*pes varus*) and joint malformations were also observed. Cleft palate was induced by injections between 9th and 13th days with the highest incidence observed for injections given on 12th or 13th day. All cleft palates were bilateral and complete but of varying widths. One cleft palate was accompanied by harelip, in which a cleft of left premaxillary bone as well as corresponding upper lip was found. Cleft palates were often associated with hematomas in subcutaneous or deeper layers of upper and lower jaws. Adactylism and brachydactylism were also usually associated with superficial hematomas. In some cases these were comparatively small, localized hematomas accompanied by congestion of adjacent blood vessels in subcutaneous tissues. In other cases large hematomas were present in deeper tissues and were often associated with absence, hypoplasia or deformity of phalanges and metacarpal bones, and disturbances of muscle development.

Subcutaneous hematomas were observed when an injection was given on or after 11th day (Table II). When pregnant mice were sacrificed in mid-pregnancy, hematomas could be recognized as early as 12 hours after injection but were most frequent in older embryos of 15-16 days fetal age. In earlier stages hematomas were usually confined to fore and hind feet but in later stages they occurred in other areas such as maxilla, mandible and tip of tail. Number and size of hematomas increased with length of time following injection. Although there appears to be a correlation between hematomas and certain malformations, other defects observed in this study such as malformed elbow joints, *pes varus* and polydactylism were not accompanied by hematomas. The association of digital deformities with hematomas resulting from caffeine administration appears to be similar to results obtained with deficiencies of linoleic acid(8) and pantothenic acid(9,10).

The placental transfer of caffeine observed in rabbits and dogs(11) and the lack of macroscopic or microscopic changes observed in the placenta in this study permit the suggestion that caffeine may have directly affected the developing embryo.

Summary. Pregnant mice were given single intraperitoneal injections of caffeine on different days of gestation. When injections were administered between 9th and 14th days, malformations, predominantly in the skeletal system, were observed. Superficial hematomas observed in vicinity of anomalies may have been concerned in development of some of these malformations. No macroscopic or microscopic changes were observed in placentae of malformed young.

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Trachoma Viruses Isolated in United States.* 1. Growth in Embryonated Eggs. (25758)

LAVELLE HANNA, ERNEST JAWETZ, PHILLIPS THYGESON AND CHANDLER DAWSON†

Dept. of Microbiology and Proctor Foundation for Research in Ophthalmology, University of California Medical Center, San Francisco

It is believed that more than 400 million people are infected with trachoma throughout the world. Diagnosis of this disease rests largely on clinical impression (conjunctivitis, with follicles particularly on upper tarsus, papillary hypertrophy, vascular invasion of cornea, pannus, scarring) and typical cytology of conjunctival scrapings with intracytoplasmic inclusion bodies. These inclusions, described over 50 years ago(1) consist of elementary and initial bodies in a matrix of glycogen. Because of the morphologic appearance of elementary bodies and their developmental stages trachoma virus was accepted as a member of the psittacosis-LGV group(2) although the virus had not been unequivocally grown in the laboratory. Tang *et al.*(3) succeeded for the first time in propagating serially trachoma viruses from patients in China, by inoculating streptomycin-treated conjunctival scrapings into the yolk sacs of embryonated eggs. Subsequently similar viruses were isolated from patients in West Africa(4), Saudi Arabia(5), and Israel(7). Trachoma is no longer a prevalent infection in the United States, except on certain Indian reservations in the Southwest. Sporadic, active cases, are occasionally seen elsewhere. We wish to report the first isolation of "trachoma viruses" from patients in the United States. To date 6 virus strains have been isolated from trachoma patients presenting typical pictures of a) acute initial adult infection, b) relapse in adult infection of 20 years' standing, and c) early infection in Apache Indian children. A preliminary report on the first of these virus strains has appeared(8).

Materials and methods. Patients: Clinical diagnoses of trachoma were made by ophthalmological examination of patients referred to us or encountered in systematic surveys of Indian reservations. *Specimens.* From active cases of trachoma conjunctival scrapings were collected in 1 ml broth-saline containing streptomycin sulfate 1-10 mg/ml and at times also polymyxin B sulfate 0.1 mg/ml. Most specimens were collected from single patients, but some field specimens were pooled scrapings from 2-6 active cases. Field specimens from Arizona were kept at −20°C to −70°C during shipment for varying periods before processing. Specimens collected in San Francisco were kept for 1 hour at 4°C before inoculation. *Embryonated eggs*: Specimens were mixed thoroughly, then 0.5 ml was inoculated into the yolk sac of 6-8-day-old embryonated eggs obtained from a commercial source. Eggs were incubated at 35°C and candled twice daily. Deaths within 48 hours of inoculation were discarded. Blind passage of surviving eggs by the yolk sac route was carried out at first between 7 and 11 days after inoculation, later regularly at 7-day in-

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† Epidemic Intelligence Service Officer, Communicable Disease Center, U.S.P.H.S.