It is probable that the observed spectral structures are to be classed as hyperfine—they arise from interactions between the magnetic moments of the nitrogen and hydrogen nuclei in each molecule with the magnetic moment of the odd electron. Despite the complexity and breadth of the spectra of their dilute solutions, the crystals of the nitro-free radicals exhibit a single sharp line.[5] The trinitrofluorenone derivative yields a rather broad, unresolved resonance peak. It is possible that the fine structure in this case, as in the case of many other free radicals, is unresolved because of its complexity. This complexity arises from the large number of nuclear moments with which the electronic moment interacts.

Although the fact that sodium metal catalyzes the polymerization of unsaturated compounds has been known for a long time, there is no general agreement concerning the mechanism of this catalysis (*8*). Two sets of experiments have been performed with styrene which indicate that the function of the sodium metal is to bring about the formation of a negative hydrocarbon free radical. This free radical ion then initiates the chain reaction involved in the polymerization, as suggested by Bolland (*9*) for the sodium-catalyzed polymerization of isoprene. In one experiment a small amount of sodium dispersion was added to a sample of styrene. After some hours the styrene had been converted to a deep-red, rubbery solid which showed a strong paramagnetic resonance absorption. It is believed that the red color and the paramagnetic absorption are due to species such as $(C_6H_5CH{=}CH_2)^-$ which were trapped in the polymer. The material retained its paramagnetic resonance absorption and its color over a period of several months.[6] In other experiments, styrene was dissolved in 1,2-dimethoxyethane and a small amount of sodium dispersion was added. A very vigorous reaction set in immediately, accompanied by the formation of a deep-orange color. As the reaction subsided, the reaction mixture became very viscous and the orange color gradually faded away. Since 1,2-dimethoxyethane, as mentioned above, favors the formation of negative hydrocarbon free radicals, it is believed that these observations are further evidence that the actual polymerization catalyst is a species such as $(C_6H_5CH{=}CH_2)^-$.

Various investigators have suggested (*10*) that abnormal growth may be explained by a free radical mechanism. Perhaps the carcinogenic activity of 20-methylcholanthrene and 1,2-benzanthracene is due to their ability to form negative hydrocarbon free radicals with mild reducing agents, whereas noncarcinogenic hydrocarbons such as naphthalene and anthracene are able to form such free radicals only with very strong reducing agents.[7]

[5] A similar phenomenon was first observed in diphenylpicrylhydazyl, dilute solutions of which have been found by Hutchison, Pastor, and Kowalsky (*J. Chem. Phys.*, **20**, 534 [1952]) to show hyperfine structure covering about 50 oersteds. The crystals, on the other hand, show a single sharp line about 2 oersteds wide.

[6] It should be noted that this experiment is not readily reproducible. In view of the competition between the formation of the catalytic species, $(C_6H_5CH{=}CH_2)^-$, and the many processes by which it may disappear, it is not surprising that the magnetic absorption phenomenon is difficult to reproduce.

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[7] A study of the relative electron affinity of various hydrocarbons is in progress in this laboratory.

Excessive Intake of Vitamin A as a Cause of Congenital Anomalies in the Rat[1]

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Congenital anomalies have been produced in animal young when the fetal environment has been influenced by chemical, endocrine, mechanical, and actinic factors (*1*). Changes in atmospheric pressure (*2*) (hypoxia) and administration of cortisone (*3*) have recently been shown to exert teratogenic effects. Similarly, deficiencies in the maternal diet of single nutritional elements such as minerals (copper [*4*] and iodine [*5*]) and vitamins (riboflavin [*6*], pantothenic acid [*7*], folic acid [*8*], and vitamin A [*9, 10*]) have induced defective offspring. Reports (*11, 12*) of vitamin A excess in the maternal diet have shown a diminished litter rate and a high incidence of fetal resorption *in utero*. In the course of our investigation of the skeletal changes of hypervitaminosis A in mature rats, it was noted that several pregnant animals produced offspring with congenital malformations. An investigation was undertaken to study this phenomenon.

One hundred and fifty female rats of the CF Wistar strain (175–200 g) were mated by exposure for 24 hr, during the pre-oestrous stage, to males of the same strain. Pregnant females were fed the standard Rockland pellet diet and water *ad lib.* From the 2nd, 3rd, or 4th to the 16th day post coitus, 35,000 IU vitamin A in 0.7 ml diluent[2] were administered daily, via stomach tube, to 100 animals in the experimental group.

[1] Aided by grants from the Loyal League for Philanthropies, Inc., New York City, and Mead Johnson Co., Evansville, Ind.

[2] The vitamin product used was an aqueous preparation containing 50,000 USP u/cc natural vitamin A dispersed in sorethitan monolaurate and water.

TABLE 1

INCIDENCE OF CONGENITAL CRANIAL ANOMALY

Animal no.	No. of offspring in litter	No. with congenital cranial anomaly	Gestation days fed 35,000 IU vitamin A
14	10	4	2–16
30	6	4	4–16
35	6	4	4–16
39	6	1	4–16
59	9	5	3–16
62	11	3	3–16
79	9	9	2–16
90	2	2	2–16
93	5	5	2–16
100	8	2	2–16
Total	72	39 (54%)	

Fifty control animals were fed 0.7 ml of the diluent. The animals were sacrificed on the 20–21st day post coitus (at or near term), and each litter was examined for gross congenital defects. In both groups most of the young were alive when removed from the amniotic sac. All were placed in fixative solution for histologic study.

Excessive intake of vitamin A from the 2nd, 3rd, or 4th to the 16th day of gestation resulted in a marked reduction in the number of litters carried to term. Of 50 control mated females, 44 produced litters, with a total of 410 apparently normal newborn, a successful pregnancy rate of 88%; whereas of 100 mated females in the experimental group, only 10 carried young to term, with a total of 74 offspring, a successful pregnancy rate of 10%. This marked pregnancy failure rate is in accord with previous reports (*11, 12*).

The incidence of gross congenital defects in the litters carried to term in the experimental group is seen in Table 1. Of 74 offspring produced in 10 litters, 34 exhibited a gross anomaly in the development of the skull and brain, a deformity rate of 54%. In the control group no anomalies were noted in 410 offspring.

The gross developmental defect apparent in each of the abnormal offspring rats is an extrusion of the brain (Fig. 1) to the external surface of the head. There is a thin membranous covering over the exposed brain tissue. Sporadic anomalies noted were macroglossia, harelip, cleft palate, and gross defects in eye development. The cranial deformity, however, was a consistent finding. It is of interest that comparable cranial anomalies (as determined by photographic similarity) have been produced in mice by other teratogenic methods such as hypoxia (*2*) induced by exposure of the pregnant animal to atmospheric pressures of high altitudes, or by the translocation effect (*13*) in the second-generation offspring following irradiation of the initial sire male.

Results of this preliminary experiment indicate that the administration of excessive amounts of vitamin A to pregnant rats produces a diminished litter rate and characteristic malformations among the surviving young.

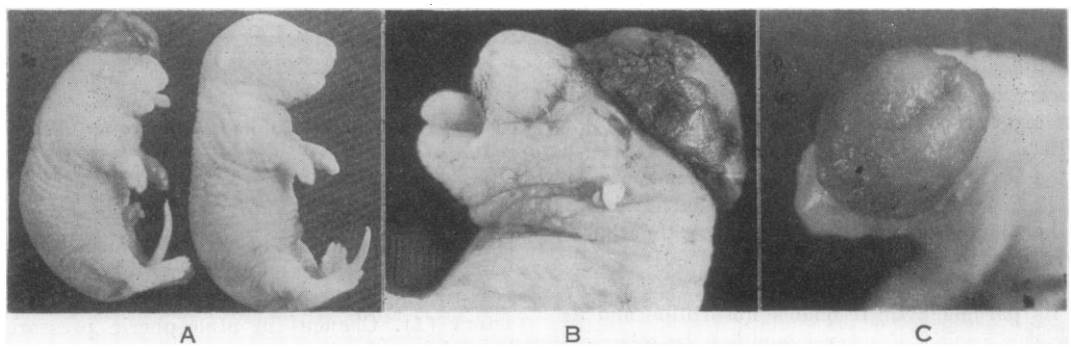

FIG. 1. *A*, lateral view of experimental and control rat at term. *B*, lateral close-up of experimental animal showing brain extruded to surface of head. Protruding tongue is abnormal. *C*, close-up view of extruded brain from above. Note longitudinal fissure of the cerebrum.

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