animals which received BGG in the complete adjuvant. Evidence was presented to indicate that the mycobacteria used to establish hypersensitivity to tuberculin may also have functioned in an adjuvant role in this case.

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A Study of Teratogenic Effects of Particulate Compounds in the Amniotic Cavity of Chick Embryos.* (31780)

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In previous studies a significant percentage of gross defects occurred after thalidomide was injected into the amniotic cavity of 3-to 5-day-old chick embryos. The defects included eye defects, encephalocele, beak defects, retarded feathering and retarded ventral closure. However, similar defects were produced by other chemically unrelated compounds, the only obvious characteristic possessed by all being a high degree of insolubility in ordinary diluents and in the amniotc fluid(1). Two compounds of the series tested were colloidal and these produced, in addition, severe distortions of the body axis and limbs. To investigate further the nature of the teratogenic action of the particles several other compounds were studied. Carbon black, easily identified in tissue sections, was used to determine the physical relationship of the particles to the defects produced. Included also was a colloidal silica of known particle size. Several preparations of polystyrene latex balls of graded size were tested in an attempt to determine the relationship of particle size to production of defects. The latex balls agglutinated in the amniotic fluid and thus were not suitable for the purpose, although they did produce the characteristic defects. Due to similarities in the effects of all the compounds, the most obvious supposition is that the developmental anomalies are related to the physical rather than to the chemical nature of the materials. There is, however, no evidence that the compounds may not also produce changes related to their chemical activities. For instance, silica particles, which are etiologically associated with silicosis are known to have specific toxic effects for macrophages which engulf them(2) whereas other particles, including those of alumina and carbon, do not have these toxic effects. In chick embryos the colloidal silica produced the same defects as colloidal alumina with certain slight dissimilarities.

Methods and materials. Materials used. Reagent grade, sugar carbon from Fischer Scientific Co. had maximum impurities of 1% sulfates (calcium, magnesium, sodium, etc.) and 1% ash (alkali metal). For injection it was ground in a tissue grinder and suspended in distilled water or saline buffered at pH 7.2. A suspension of colloidal silica (Ludox®) from Du Pont Co. contained 15% SiO_2, 1% Na_2O (titratable alkali), 0.001% NaCl and 0.003% Na_2SO_4. The silica particles were dense, nonporous spheres approximately 7 mμ in diameter. They remained dis-

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TABLE I. Survival and Incidence of Abnormal Development in Chick Embryos Inoculated with Carbon Suspensions into the Amniotic Cavity at 5 Days Incubation.

mg contained in 0.05 ml diluent per egg	Total eggs	Embryos surviving to incubation day: 8	15	Total abnormal of those surviving 8 days or more No.	(%)
Carbon 0.1	73	38	10	19	(50)
" 0.01	52	30	13	5	(17)
" 0.001	51	29	9	1	(3)
Control	72	51	27	0	

crete and maintained particle size on dilution in water or saline. The pH was 8.5 with minimum stability range at pH 5-6. For injection the "Ludox" was diluted in water or saline to the required concentration. Broth cultures were made of all materials inoculated. The preparations rarely showed bacterial contamination even though sterilization procedures and addition of antibiotics were generally avoided to ensure stability and purity of the preparations. In some experiments carbon suspensions were autoclaved but results were the same whether or not this procedure was followed.

Preparation and inoculation of eggs. Fertile eggs from White Leghorn flocks or from Meatline hens mated to New Hampshire roosters were incubated in a forced draft incubator at 37.5°C. Materials were injected, according to methods previously described(1) into the amniotic cavity of eggs incubated for 5 days. Each received 0.05 mg of the diluent containing calculated amounts, ranging from 0.001 mg to 0.25 mg, of the experimental materials. Controls from the same incubation setting received saline or distilled water with the pH adjusted to that of the experimental material.

Harvesting and examination of embryos. In the initial experiments with carbon and silica, the embryos were injected, then observed daily through the shell openings and the dead embryos removed for gross examination. Surviving embryos were sacrificed at 15 days of incubation. Incidence and description of defects were tabulated for all embryos surviving to the 8th day of incubation or longer. In another series of experiments, embryos were injected with carbon suspensions and sacrificed at intervals of 1, 3 and 7 hours and thereafter at daily intervals up to the 8th day after injection. These were examined grossly, then processed by standard histological techniques for microscopic study. Sections were stained with hematoxylin and eosin, or in some instances, the periodic acid-Schiff stain.

Results. Observation of gross defects. Embryos receiving carbon suspensions showed a significant percentage of striking defects of the types previously observed with thalidomide (Table I). In order of incidence, the defects included retarded lid development or exophthalmia, encephalocele or exencephaly, external edematous blebs, ectopic viscera, retarded feathering and crossed or short upper beak. All abnormal embryos had eye defect or encephalocele or both, predominantly on the left side. This asymmetry is attributed to the fact that carbon aggregates sink to the bottom of the amniotic cavity where the left side of the head lies at the stages injected.

The colloidal silica showed no indication of clumping or settling in the amniotic fluid. It also produced a significant percentage of defective embryos at dosages indicated in Table II. The types and incidence of defects were similar to those observed with the carbon but with the addition of severe axial distortions and correlated limb distortions similar to those previously observed with colloidal alumina(1). The silica-treated embryos frequently showed fusion of the appendages to the body wall with concomitant malformations of the affected limbs. These fusions had not been observed with the colloidal alumina.

Microscopic study of carbon-induced defects. In this series of experiments, eggs were injected at 4 to 5 days with amounts ranging from 0.1 to 0.25 mg of carbon per egg, the larger amounts making it easier to locate particles in tissues without extensive serial sectioning. A total of 53 embryos were studied,

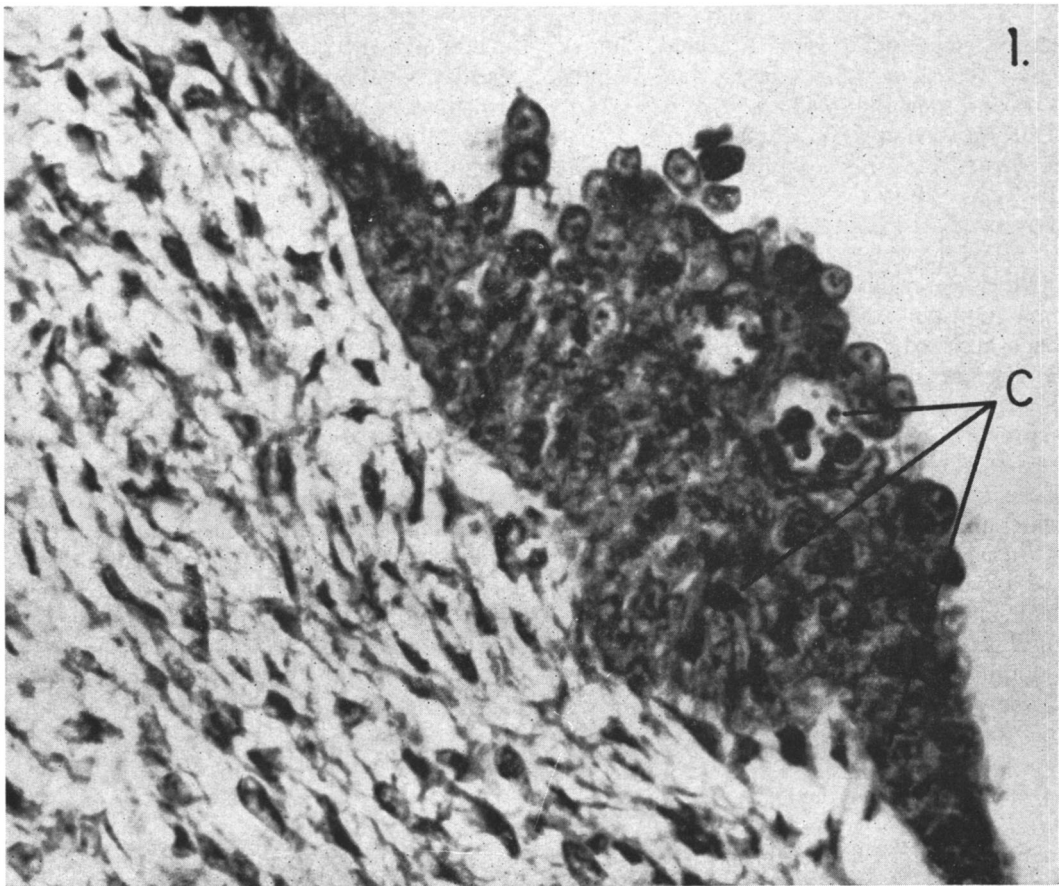
TABLE II. Survival and Incidence of Abnormal Development in Chick Embryos Inoculated with Colloidal Silica into the Amniotic Cavity at 5 Days Incubation.

mg contained in 0.05 ml diluent per egg	Total eggs	Embryos surviving to incubation day: 8	15	Total abnormal of those surviving 8 days or more No.	(%)
Silica 0.82	44	25	14	10	(40)
" 0.082	164	114	64	59	(52)
" 0.0082	44	30	20	5	(17)
Control	110	100	78	0	0

3 to 6 embryos sacrificed at each time interval after injection.

On gross examination the carbon was at first observed floating in the amniotic fluid but within a few hours it began to adhere to tissues and, as the days passed, it disappeared from view in most embryos. Microscopic examination showed that carbon began to enter tissues of the amnion and ectoderm of the embryo by 7 hours after injection. By 1 day small particles of carbon had been taken up by cells of the amnion and aggregates of carbon had been incorporated into the amniotic tissue by outgrowth of cells. Carbon was also present in the integumental ectoderm of the embryo itself.

The mechanism by which the carbon entered embryonic tissues is not clear but phagocytosis *per se* did not appear to be a factor. Particles were sometimes located within ecto-



Microscopic sections of chick embryos injected into amniotic cavity at 5 days incubation with 0.1-0.25 mg of carbon (C).

FIG. 1. Area of localized hyperplasia in cephalic ectoderm, 4 days after injection. × 460.

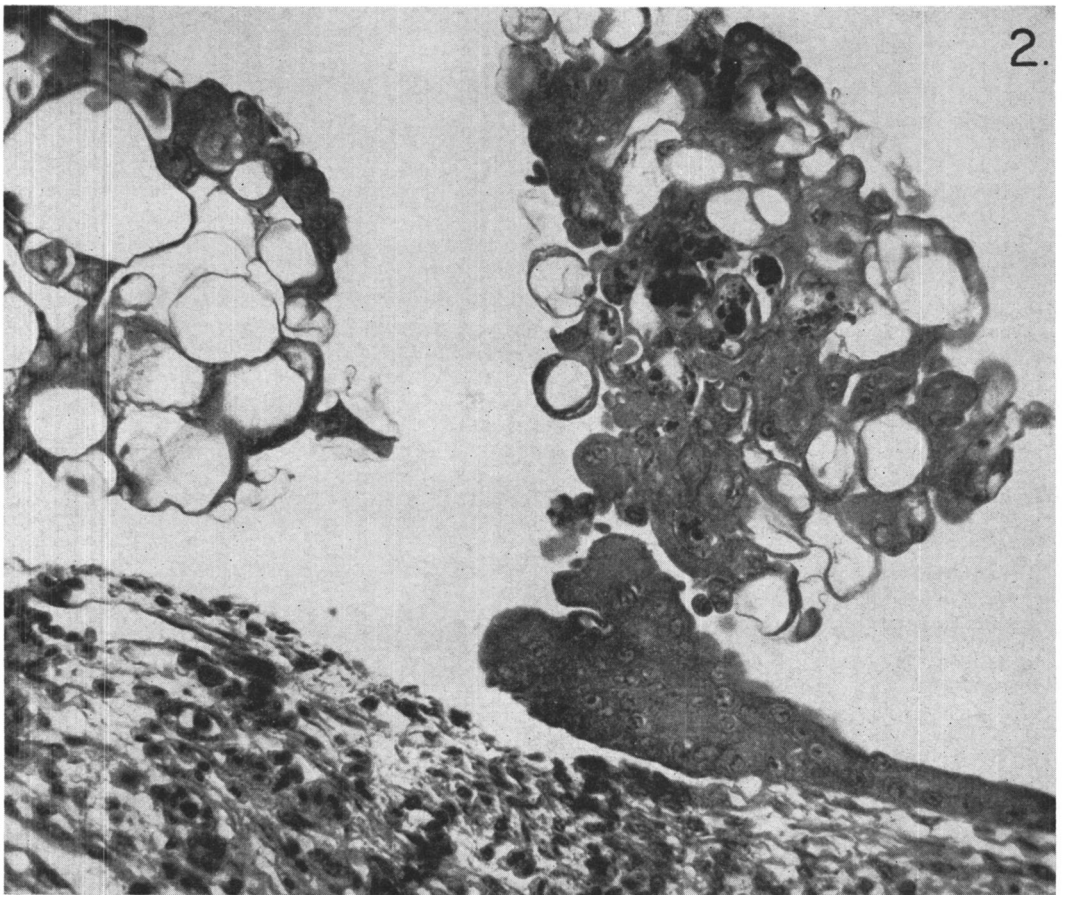


FIG. 2. Hyperplasia and sloughing of vacuolated tissue of ectoderm over the optic lobe, 7 days after injection. × 230.

dermal cells but were most commonly observed within large tissue spaces, among damaged cells and sometimes between cells which appeared normal. Localized reactions of ectodermal tissues in the vicinity of carbon included: a) sloughing of outer ectodermal cells, some with and some without carbon attached; b) damage to some cells in the vicinity of carbon as indicated by fading of cell membranes and nuclei; c) marked localized hyperplasia of the ectoderm with consequent disorganization of normal cellular relationships (Fig. 1); d) appearance of large clear spaces in the tissues (Fig. 2); e) changes in cell characteristics such as increased size, changes in shape and increased affinity for eosin. Necrosis and sloughing of the ectoderm resulted in its complete destruction in some areas where the outer cells of the mesoderm then joined to form a covering for the subjacent tissues. Although the edges of such areas were usually bounded by thickening of the remaining ectoderm, there was no evidence during the period of observation that the ectodermal cells migrated to cover the exposed mesoderm. In some areas fusions occurred between amniotic membrane and the integument.

At 1 to 2 days after injection, the carbon began to enter the mesoderm. Although carbon could presumably enter through the denuded areas, it was, apparently, also able to pass through ectoderm into the mesoderm. In many sections carbon was observed in undestroyed body ectoderm and in the underlying mesoderm. No characteristic reaction of the mesoderm to the carbon was observed but the tissues in the affected areas always become thinner with carbon located at pro-

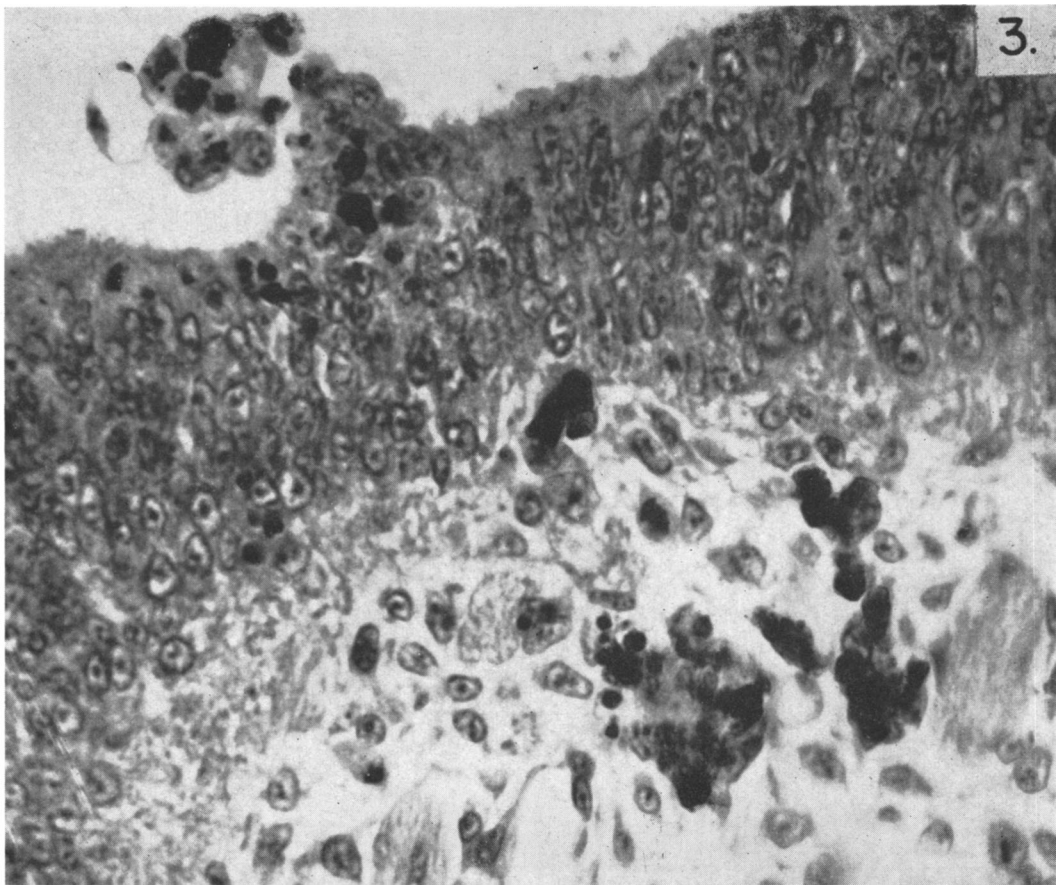


FIG. 3. Nasal epithelium in area of superior concha shows carbon particles in sloughing cells, nasal epithelium and subjacent mesenchyme, 5 days after injection. × 460.

gressively deeper levels within the tissues. No inflammatory reaction occurred in the early days after injection, no walling off of carbon by connective tissue and no obvious invasion of the blood stream. There were occasional degenerating cells in the vicinity of carbon particles and rarely a cell with phagocytosed carbon was evident. Some areas of hemorrhage and edema were seen.

Initially it was considered that damage to the body ectoderm might be due to friction of particles against the cells as the embryo moved about in the amniotic cavity. However, sections through the nasal cavities, which are protected against such frictional movements, provided evidence against this explanation. Carbon particles were frequently observed within the nasal epithelium where the effects were much the same as those in the integumental ectoderm, with necrosis and sloughing of cells, disorganization of normal cellular arrangements and passage of carbon through the nasal epithelium (which is much thicker than the integumental epithelium) into the underlying mesoderm (Fig. 3).

Wherever sufficient carbon was present in the tissues, the structures developing in or near such tissues were abnormal. Thus, sclerotic cartilages developed irregularly in the vicinity of carbon, some areas being interrupted by undifferentiated mesenchyme while others appeared thicker than normal (Fig. 4). Feather follicles were frequently absent in the cephalic region (Figs. 5, 6, lids and nictitating membrane were absent or retarded and the cornea was thin or absent. In affected areas of mesoderm, the frontal and squamosal bones were retarded in growth, failing to extend over the cranium (Fig. 6).

Eventually most of the carbon was located

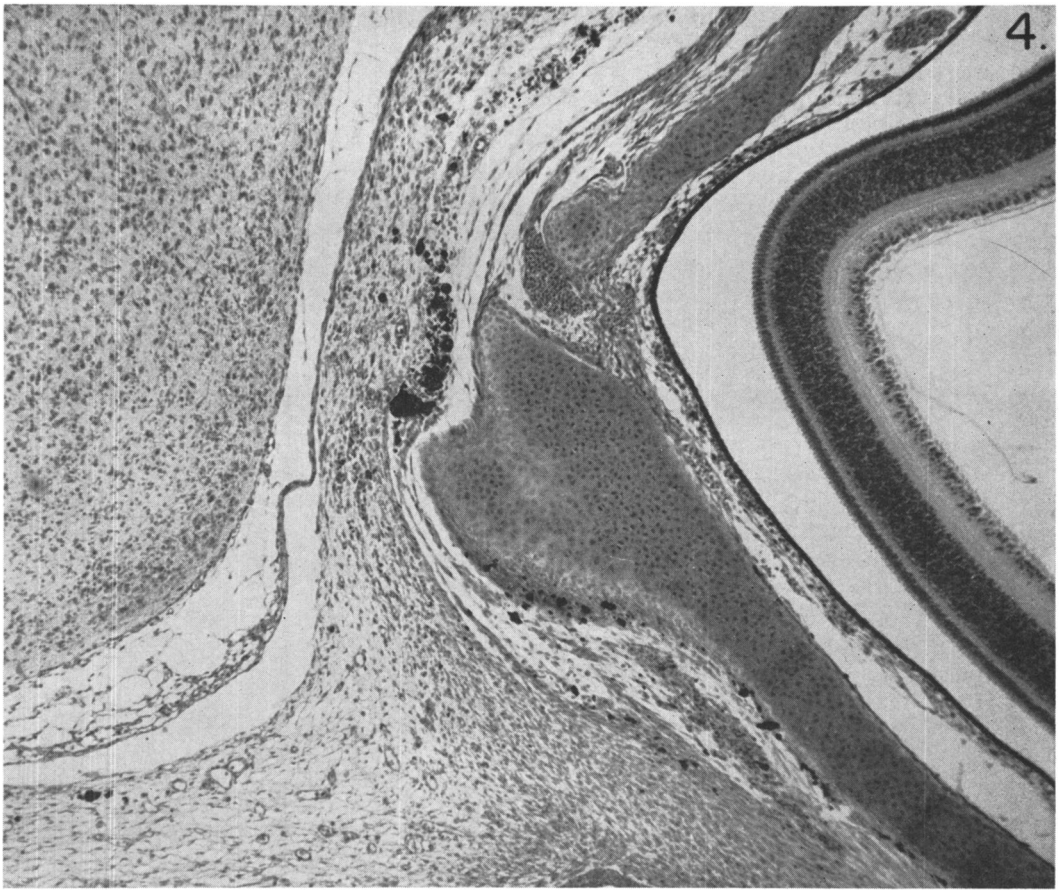


FIG. 4. Shows accumulation of carbon in mesenchyme between left eye and cerebral hemisphere, with abnormalities of the sclerotic cartilage 7 days after injection. × 45.

deep within mesodermal tissues, particularly between the cerebral hemispheres and the interorbital septum, adjacent to or within sclerotic cartilages (Fig. 4), mesenchyme of the ventral abdominal wall and in tissues of extraembryonic membranes including amnion, chorion and yolk sac. Within the experimental period, carbon was not found in tissues of brain, lens, retina or in the visceral organs. However, during the latest stages examined, phagocytosis of carbon particles was increasingly seen in cells within the body cavities and occasionally in cells of the mesoderm over the brain.

Discussion. The similarity of teratogenic effects of colloidal silica to those of colloidal alumina (Du Pont's "Baymal") calls for a comparison of some of the physicochemical properties of the two compounds. The particles of the colloidal alumina were filamentous, ranging from 100 mμ to 300 mμ in length compared to the spherical silica particles only 7 mμ in diameter. Silica releases a small amount of silicic acid which is believed to be involved in its ability to destroy macrophages(2). Recent studies also indicate that silica can interact with proteins and phospholipids and lyse red blood cells (3). In contrast, the colloidal alumina does not have these effects and has been shown to be highly nontoxic in animal studies (Du Pont's manual on "Baymal"). It should not have been too surprising to find the silica preparation causing teratogenic effects but there is no ready explanation for the severe damage to cells and tissues by such materials as alumina or carbon. In fact carbon has been used as a marker in cells of early chick blastoderms to study cell movements. In some of these studies the investigators did report a

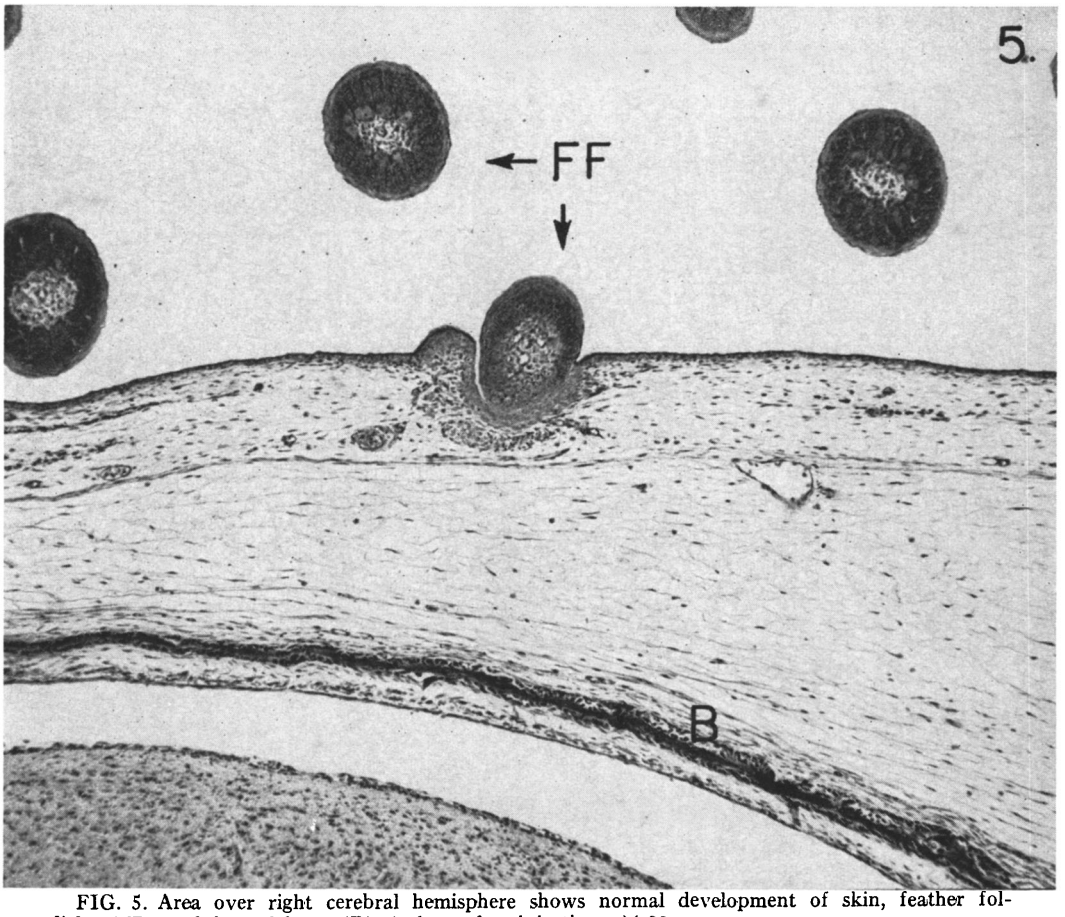


FIG. 5. Area over right cerebral hemisphere shows normal development of skin, feather follicles (FF) and frontal bone (B), 7 days after injection. × 90.

high incidence of abnormalities, including spina bifida and eye defects(4) but they did not report observing carbon in association with the defective structures. In the present experiments the amount of carbon used was undoubtedly greatly in excess of that used in the marking experiments.

It is generally agreed that most cells of the early chick blastoderm possess phagocytic properties(5,6) but these largely disappear from ectodermal and endodermal tissues by 2 to 3 days of age, except for cells of the amnion and certain epithelia(6). Our observations confirm this in that the carbon particles did not appear to be phagocytosed by ectodermal cells of the integument. Adherence of the carbon and penetration into the embryonic tissues in our studies is undoubtedly related to the immature state of the tissues whereas in more fully differentiated skin, the layers of cornified epithelium prevent entry of most foreign material. Natural stickiness of the cell surfaces may have been involved in the initial adherence of the particles but the mechanism by which they penetrated the tissues and became located deep within mesoderm is not known.

Destruction of surface epithelium clearly played an important role in the production of some of the defects. Since this epithelium participates in development of eyelids, cornea and feather follicles, its continual destruction could be expected to interfere with these structures. It also seems probable that the presence of carbon within the mesenchyme interferes with the formation of bone and cartilage in the area. It has been shown that localized edema or small hematomas can cause malformation of the developing structures in which they occur(7). Carbon also produced areas of hem-

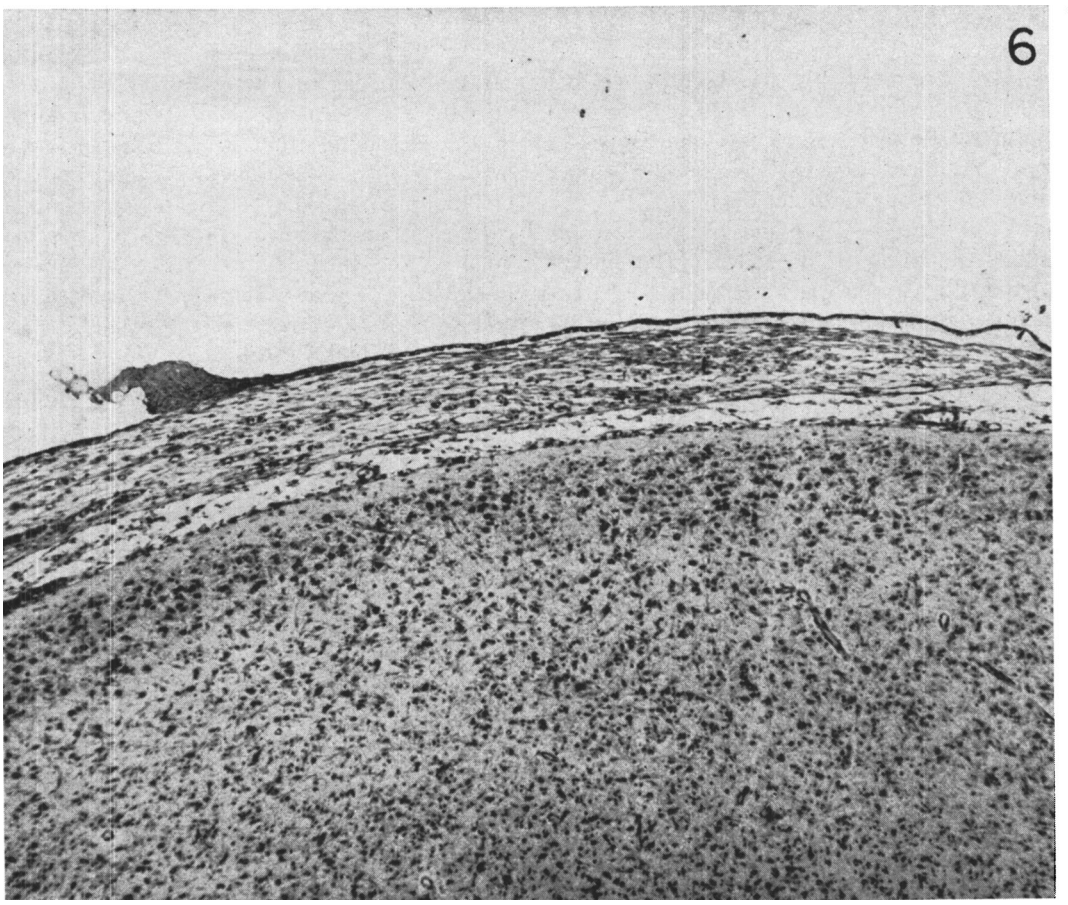


FIG. 6. Area over left cerebral hemisphere of the same embryo as that in Fig. 5, shows failure of normal development of skin and frontal bone and absence of feather follicles. Particles of carbon in epithelium and mesenchyme are too small to identify at this magnification. × 90.

orrhage and edema, but it seems possible that the foreign particles themselves could also interfere with development. Since it has been repeatedly demonstrated that interaction between tissues is necessary for normal development(8) the mechanisms involved in these defects may be quite complex.

Distribution of the carbon particles in tissues with resulting defects can probably be correlated with similar defects caused by other particulate materials and with thalidomide injected into the chick embryo under similar circumstances(1). There is, of course, no evidence that these phenomena can be related to thalidomide-induced defects in the human or with other birth defects. However, the severe defects observed with all the particulate materials and the ability of the carbon to pass from one tissue to another by means other than the circulation or transportation within cells, suggest that it might be worthwhile to consider whether foreign particles could, under naturally occurring conditions, gain access to embryonic tissues. Whether chemical compounds may change under *in vivo* conditions to become deposited in crystalline form in embryonic tissues is not known. During investigations of the mechanisms of teratogenic action, it seems reasonable to consider this possibility along with studies of biological activities involved.

Summary. Silica, which is known to have certain toxic properties, was injected in colloidal suspension of minute particle size into

the amniotic cavity of chick embryos. It produced defects very similar to those previously observed with the highly nontoxic colloidal alumina. Carbon particles also produced striking defects of the same types as those produced by thalidomide and other noncolloidal but insoluble compounds which have been studied previously. Microscopic studies of tissues from chick embryos inoculated with carbon particles indicated that the particles entered the integumental ectoderm where they caused severe necrosis and sloughing of cells as well as marked localized hyperplasia. Following this the carbon entered the mesoderm, either through the denuded areas or, as observed in many instances, by passing through the ectoderm into the subjacent mesoderm. In the mesoderm the particles became located at progressively deeper levels, in some cases penetrating cartilagenous structures. Studies of the distribution of the carbon within the embryonic tissues indicated that defects occurred in structures developing in or near tissues in which the particles were located. Continual destruction of surface epithelium of the cephalic region which plays an important role in the development of skin, feather follicles, eyelids, nictitating membrane and cornea, resulted in defective development of these structures. When carbon was present in areas of the mesoderm from which bone or cartilage developed these structures were retarded or abnormal. Since the greater amount of carbon accumulated in the cephalic region (due to the tendency of carbon aggregates to gravitate toward this region) the frontal and squamosal bones and the sclerotic cartilages were chiefly affected.

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Metrecal-Induced Changes in Human Saliva.* (31781)

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Feeding a liquid diet of Metrecal to rats produces structural and functional changes of the salivary glands which are believed to represent atrophy of disuse(1,2). The liquid consistency of the diet results in reduced masticatory reflexes and the atrophy is related to consequent reduction in stimulation of the salivary glands rather than any nutritional factors. The structural and functional changes that result are particularly marked in the parotid and consist principally of acinar-cell atrophy and alteration in the water, protein, and electrolyte composition of the secretion. Furthermore, all changes may be completely reversed by reinstituting a diet of solid food. This evidence suggests that activity is an important determinant of the status of salivary glands in rats, but its role in maintenance of human salivary glands has not yet been determined. Consequently in the present investigations the effects of liquid diet on human salivary glands were examined. Since direct examination of the gland was not feasible, only the secretion from such glands was analyzed for changes indicative of atrophy. The results show that an exclusive diet of liquid Metrecal alters the secretion of water and

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