Production of Meningoceles and Cranioschisis in Chick Embryos with Lead Nitrate.* (19340)

E. M. Butt, H. E. Pearson, and D. G. Simonsen.

From the Los Angeles County Hospital and the Departments of Pathology and Medical Microbiology, University of Southern California School of Medicine.

Since finding abnormal amounts of lead and mercury in some cases of congenital disease of the newborn(1) an attempt was made to induce similar changes in chick embryos with lead, mercury and copper salts.

Various substances introduced into the fertile egg may cause abnormalities in chick embryos. Hammett and Wallace(2) noted that small quantities of lead affected the head and optic anlagen and body growth was retarded. Gray(3) found that lead chloride had an apparent effect on the central nervous system. Later Catizone and Gray(4) noted various central nervous system alterations after the injection of lead chloride, including destruction of the anterior end, failure to close and the production of a "sinuous nervous system". Hansborough(5) reported similar changes in chick embryos following the injection of 2 cc of 1% nicotinic acid. His conclusions were similar to those of Taylor, *et al.* (6) who used pantothenic acid. Franke, *et al.* (7) used selenium to produce monstrosities in chick embryos. Insulin and other chemicals were used by Landauer(8). Hunt(9) noted a variety of abnormal morphological types in chick embryos developing from eggs injected with 2 M sucrose. The principal affected areas were the nervous and circulatory systems. Newcastle virus infection of embryos resulted in defective development of the lens and auditory sac(10).

Procedure. Since the appearance of meningoceles was so striking in the leaded embryos the experiment was confined to observation of this lesion. Occasionally other anomalies such as crossed beaks, absence of eyes or heterotaxy were noted in the uninjected controls, anion controls, copper-or-mercury-treated embryos. However, in no instance was a meningocele seen except in lead-treated embryos. White Leghorn eggs were incubated at 39°C in a moist atmosphere. 0.1 cc of solution was injected into the albumen. Lead nitrate and mercuric bichloride were made up in normal saline to contain 0.5-1% lead or mercury. Copper sulfate containing 1-2% Cu was used. Control eggs were injected with sodium salts of chloride, nitrate and sulfate to test for anion effects.

Results. The results of injections of Pb, Hg and Cu are listed in Table I. A total of 715 eggs was used, of which 78% were

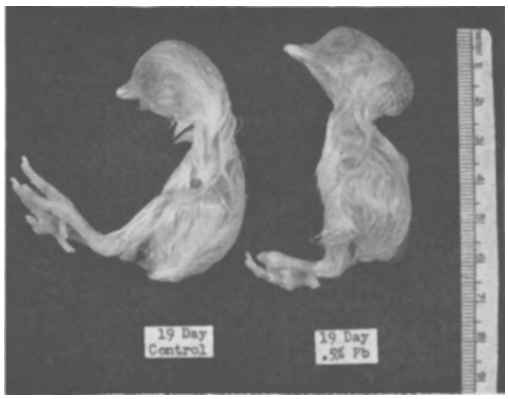

FIG. 1. Meningocele in leaded embryo.

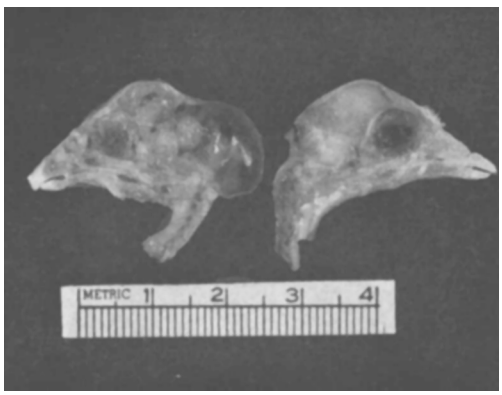

FIG. 2. Sagittal sections of heads of 19 day chick embryos (Fig. 1). Meningocele seen in leaded embryo at left. Saline control at right.

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TABLE I. Survival of Eggs Injected Before Incubation.

Inj. cation, mg	Inj. eggs	Fertile eggs	% dead before 13th day	Incubation day of examination									Alive at termination of exp.	Meningoceles
				13	14	15	16	17	18	19	20	21		
Pb 1	74	66	79	1/1	1/2	1/1	2/2	4/9		1/1*			7	10
Pb .75	144	109	58	1/4	5/14		1/2		0/1	0/5	3/19	1/3	3	11
Pb .5	24	18	61		0/3				1/4				4	1
Hg 1	60	45	80	1					8				8	0
Hg .75	36	32	53		1				14				12	0
Hg .5	24	15	53								7		7	0
Cu 2	24	19	68		1					2	3		3	0
Cu 1	41	27	74		1				3		3		6	0
Totals	715	344												
% fertile—78														

* Numerator—No. with meningoceles. Denominator—No. examined.

fertile. It is seen that mortality of the chick embryos in the first 13 days of incubation is high and probably is related to toxicity of metallic ions. The meningocele lesion was not grossly apparent until approximately the 13th day of incubation. The eggs were opened from the 17th to the 21st day and no attempt was made to allow hatching. Most of the embryos reaching these days of development were alive, and it can be seen the mortality was extremely high even after the 13th day. The weight of the leaded embryos averaged less than the control embryos. The meningoceles consisted of protrusions of the arachnoid and dura covered by skin. The space was occupied by a thin fluid that appeared to be under slight pressure. These lesions were present in the occipital regions and rarely in the region of the anterior superior portions of the skull. One embryo had 2 such protrusions of the meninges. Lack of bone development in the region of the meningocele was obvious (Fig. 1 and 2). Examination of microscopic sections revealed degenerative changes in the cerebellum and other portions of the brain, due perhaps to pressure from the meningocele and to toxic effect of the lead ion. In experiments not recorded in Table I no meningoceles were seen in embryos from 152 eggs injected with lead ion concentrations of from 0.01 to 0.05%.

Control eggs were inoculated to test effects of the anions used. A total of 185 eggs were injected, each with 0.1 cc of sterile 0.85% NaCl. 81% were fertile and 12% died before the 13th day of incubation. 77% of the embryos survived until the eggs were opened on the 17th to the 20th day of incubation. In most instances development was normal. Rarely was a crossed beak or deformed eye noted. The mean average weight of this group was higher than that of the metal exposed embryos opened on corresponding days. Forty-two eggs, of which 81% were fertile, were injected, each with 0.1 cc of 2% Na_2SO_4. The mortality was 33% in the first 13 days. This was definitely higher than in the saline injected eggs but less than in the metal groups. No gross abnormalities were found in those embryos surviving the 13th day of incubation.

Forty-seven eggs were injected with 0.1 cc of 0.8% $NaNO_3$. 20% of the embryos were dead before the 13th day. Three died on the 15th day and 28 were alive on the 17th day. No gross abnormalities were seen.

Discussion. The production of developmental anomalies by exogenous substances is not a new finding and Mall(11) suggested that most human monsters resulted from such agents. However, during the past decade considerable interest has been stimulated by observations of the relationship of congenital defects and German measles(12). More recently unusually large amounts of lead were found in defective newborn infants(13).

In the present study it is of interest that lead produced grossly defective chick embryos. Copper and mercury did not produce meningoceles although sufficient in quantity to produce death in a similar number of embryos before the 13th day. Eggs from two unrelated flocks of White Leghorns were used and the results were the same. Meningoceles were not present in the non-leaded controls and did

not appear in the copper, mercury or anion injected controls, but appeared in equal numbers in the leaded chicks. It would seem that lead has a specific effect in the production of this particular lesion in the occasional embryo. We do not know if the defect occurs spontaneously. In the human the lesion has been considered to be of hereditary origin since it has appeared in families of the involved infants or embryos(14).

It is quite probable that meningoceles in chick embryos are a late development of the "open nervous system" described by Catizone and Gray(4). They found the lesion most frequently when lead was injected into the subgerminal cavity at 18 hours incubation. Obviously the ion injection in the albumen would be subject to greater variation in its quantitative relationship to the embryo. It is possible that a larger number of meningoceles could have been produced by injection at a different stage of incubation or by exposing the embryo more directly through subgerminal cavity injection. That the lesion could be produced even relatively late in embryonic development is evident from the observation of meningoceles in 3 of 29 embryos which developed in eggs injected with 0.75-1 mg of lead on the 5th day of incubation.

Conclusion. (1) Injection of lead into the albumen of fertile eggs may result in the production of meningoceles in chick embryos. (2) Copper and mercury ions were noted to be as toxic as similar quantities of the lead ion, but meningoceles were not seen in the embryos surviving 13 days incubation. (3) Sodium salts of anion used in the metal experiments failed to produce meningoceles.

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Failure of Nembutal to Block Ovulation in the Hen. (19341)

J. W. BASTIAN AND M. X. ZARROW.

From the Department of Biological Sciences, Purdue University, Lafayette, Ind.

Data have recently been accumulated indicating that the nervous system participates in the release of tropic hormones from the pituitary gland(1,2). Specifically it has been reported that the hypothalamus is involved in the release of the luteinizing hormone (LH), thyrotropic hormone (TSH) and adrenocorticotropic hormone (ACTH) from anterior pituitary gland(3-5). Experiments involving electrical stimulation in the rabbit have shown that ovulation occurs much more readily upon stimulation of the hypothalamus than upon direct stimulation of the hypophysis(6,7). Brooks, Beadenkopf, and Bojar(8) reported ovulation in the rabbit following injection of picrotoxin, a central nervous system stimulant. Pre-treatment with phenobarbital prevented the ovulatory-inducing action of the picrotoxin(9) and since barbiturates are known to have a selective action on the hypothalamus this would further tend to involve the diencephalon in the release of LH. Everett and Sawyer(10) have shown in the rat that Nembutal anesthesia between 2 and 4 p.m. on the