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## DISLOCATION OF THE HIP INDUCED BY INSULIN IN THE CHICK EMBRYO

*By*

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The mode of origin and the morphological development of congenital dislocation of the hip during embryonic life have been the objects of various theories and our knowledge of their aetiology is still unsatisfactory. At the present time it is fairly clear that the cause may be both genetic and due to an unfavourable environmental factor affecting the foetus during its early development (1).

The possibilities of studying the development of the various congenital deformities during embryonic life are very limited in the human. However, such possibilities are much better in some experimental animals, *e.g.* the chick, where it is easy to induce various skeletal anomalies by experimental teratogenesis and thus obtain experimental material during different foetal stages. The production of skeletal deformities by the injection of insulin into the egg of the chick was first described by *Landauer* 1945 (2). In a paper in 1952, *Duraiswami* (3) showed that various particular skeletal deformities could be induced in chicks by insulin injections at different periods of incubation and he drew up a plan of the localization of skeletal deformities depending on the time of injection. In his material, *Duraiswami* also demonstrated a dislocation of the hip in a chick which was injected with 6 IU insulin on the sixth day of incubation. No detailed description of the frequency and development of dislocation of the hip seems to have been given by *Duraiswami* or by any other author.

The aim of the present investigation was, in the first place, to find out the possibility of reproducing a dislocation of the hip similar to that described by *Duraiswami*. If this proved possible, a material from different stages of embryonic life could be obtained so that the sequence of morphological deformities could be followed.

## MATERIAL AND METHODS

Eggs of the White Leghorn were used. The material was divided into three series. In the first series 1379 eggs were each injected with 6 IU or 0.25 mg. crystalline insulin in physiological saline solution on the sixth day of incubation. Two series were used as controls. In the first of these, 111 eggs were each injected with 0.05 ml. sterile saline solution at the same time after incubation. In the second, 598 completely untreated eggs were incubated and examined. The material is shown in Table 1.

TABLE 1

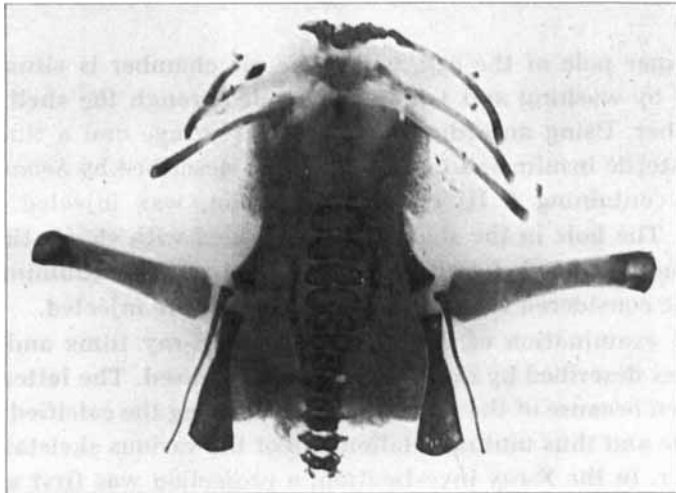
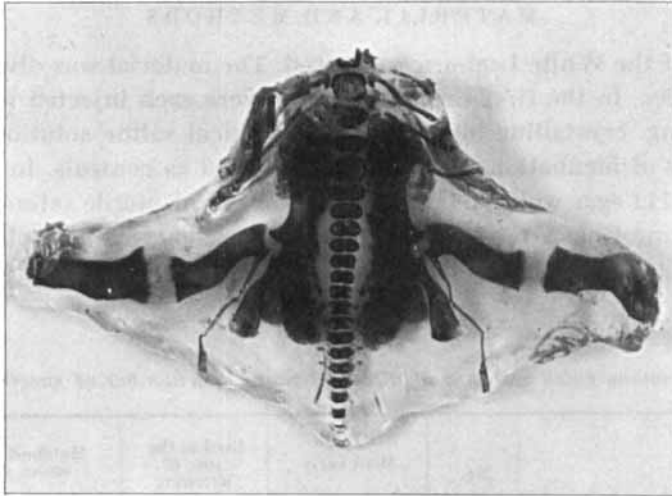
*Mortality among chick embryos at different times and number of survived chicks.*

	No.	Died early		Died at the time of hatching		Hatched alive		Survived at least 1 week No.
		No.	%	No.	%	No.	%	
Insulin injected .....	1379	1106	80	222	16	51	4	29
Saline injected .....	111	32	29	66	59	13	12	13
Untreated .....	598	157	26	313	53	128	21	128

The larger pole of the egg, where the air chamber is situated, was sterilized by washing and a hole was made through the shell into the air chamber. Using an ordinary tuberculin syringe and a fine needle, 0.05 ml. sterile insulin solution, prepared as described by *Sevastikoglou* (4) and containing 6 IU crystalline insulin, was injected into the allantois. The hole in the shell was then sealed with sterile tinfoil and incubation continued. Before injection every egg was illuminated and only those considered to have a living embryo were injected.

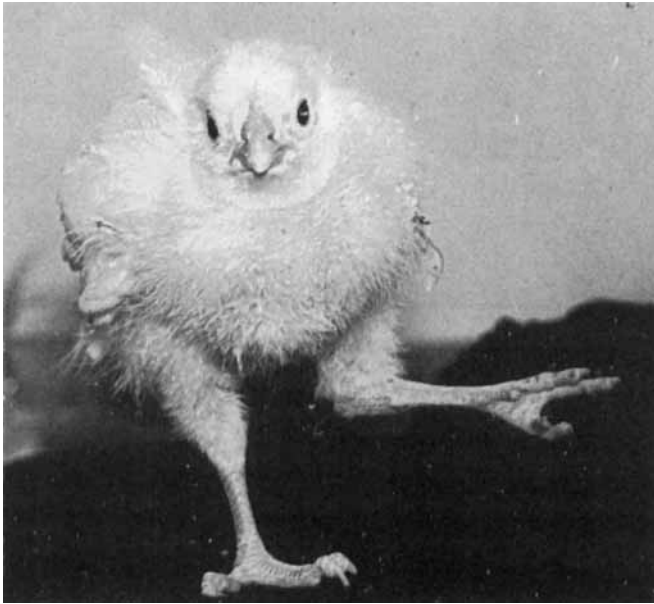
During examination of the material, both X-ray films and alizarin staining as described by *Couch et al.* (5) were used. The latter method was chosen because of the possibilities of studying the calcified skeleton as a whole and thus mutual relationship of the various skeletal parts to each other. In the X-ray investigation, a projection was first used with the specimen in the prone position and legs approximately 90° abducted. Later the specimen was X-rayed in the supine position with the legs only 30° abducted in an attempt to show up latent dislocations.

To study possible changes in the hip region, serial sections of the pelvis and hips were taken in ten randomly chosen insulin treated and ten random untreated fetuses, which died at hatching. The sections were stained with haematoxylin-eosin.



*Fig. 1.*

Alizarin stained pelvic sections of chicks, dead at hatching, the upper treated with insulin, the lower untreated. Note the general retardation, with bent, short and broad leg bones in the upper picture. The hip joints are normal in both sections.



*Fig. 2.*

Three weeks old insulin treated chick with a clinically suspect left-sided dislocated hip. Note the laterally rotated left leg giving no support.

#### RESULTS

Of the 1379 insulin treated chick embryos 1106 (80%) died soon after the injection. Of the remainder, 222 (16%) survived until hatching but died then, while only 51 (4%) hatched alive. Of these 51 only 17 survived at least two weeks, when the inorganic component of bone in the hip joint is high enough for the X-ray films to be assessed with a high degree of certainty.

All the specimens in the insulin treated series which survived as far as hatching were examined with both X-rays and alizarin (Fig. 1).

The insulin treated embryos which died at hatching did not show any definite dislocation. On the other hand, they showed a strikingly large number of general skeletal deformities, with short, broad and bent bones. In addition were found quite a number of local deformities like club foot and some cases with anomalies of the beak and anophthalmos. A certain general retardation was also noticed among the survivors but no manifest deformities. Among these were found four, three weeks old chicks with suspected unilateral dislocation (Fig. 2). These chicks only



*Fig. 3.*

Two three weeks old chicks with left-sided dislocation of the hip.  
The right hip is normal in both.

bore weight on one leg when walking, while the other leg showed an abnormal mobility of the hip, was considerably rotated laterally and gave no support. However, X-ray examination confirmed a dislocation in only two of them and these were definite dislocations of the left hip (Fig. 3).

Of the control embryos treated with saline, 32 (29%) died early, while 66 (59%) died soon after or at the time of hatching. Only 13 (12%) survived. Of the survivors, five were killed and examined after a week, four after two weeks and four after three weeks. All in this series were examined by X-rays but only 41 were stained with alizarin. There was no sign of dislocation of the hip in a single case. Furthermore, inspection showed normal development for the age in all and no signs of the above mentioned skeletal deformities could be observed either on X-ray examination or following staining with alizarin.

In the other control series there was a total of 598 completely untreated embryos. Of these, 157 (26%) died early in the incubation period and 313 (53%) just before or during hatching, while 128 (21%) hatched alive. Apart from those dying early, all in this group were X-rayed and studied by staining with alizarin (See Fig. 1, lower picture). No case of dislocation of the hip or more serious skeletal deformities was encountered in this group.

In the material which was examined histologically, normal cartilaginous structure was found without signs of special changes in the skeleton or surrounding tissues in both the insulin treated and untreated foetuses. In addition, bone formation was normal and similar in both groups without any definite signs of retardation in those treated with insulin.

## DISCUSSION

The results show that the possibility of producing dislocations of the hip as discussed in the introduction does exist to some extent. However, the methods used for recording deformities have been found inadequate, so that possible dislocations of the hip during different embryonic stages could not be shown. Only X-ray examination gave certain diagnostic information and this was only positive in chicks at least two weeks old where the calcification of the hip skeleton makes it possible to assess X-ray films. There were two such dislocations among the 17 chicks of at least two weeks of age and in two others there were clinical signs of dislocation, invisible on X-ray, probably because of unsatisfactory projection and possibly because reposition occurred by manipulations during the X-ray examination. Even if the material is much too small to allow statistical conclusions, the percentage occurrence of dislocation diagnosed by X-rays or by clinical examination in this series can be considered to be as high as 11.8% and 23.5% respectively. It is also impossible to exclude further possible dislocations in the larger part of the material, which because of the badly calcified skeleton could not be diagnosed with the methods used.

Two series served as control material. The former was to show if the injury for the embryos at the time of injection and the increase of pressure in its environment could be of importance for the development of malformation. The latter was to study the possible occurrence deformity in a normal untreated series. The untreated and saline treated embryos showed no tendency to malformation generalized or localized to the hip.

In addition the results indicate that insulin injection in chick embryos during the sixth day of incubation produces both generalized chondrodystrophic skeletal deformities and isolated deformities in the hip joint. It is known (*Landauer, Duraiswami, Sevastikoglou* (1, 2, 6)) that chondrodystrophy is produced when insulin is injected during the fourth day of incubation. On the other hand it is known that the biological development and differentiation of the embryo can differ widely from its chronological age (*Lillie* (7)).

It can therefore be assumed that in these experiments insulin affected both embryos which in spite of their chronological age were in a much earlier stage of development when the generalized malformations were induced, and also embryos whose biological development agreed well with their chronological age when dislocation of the hip occurred. The latter chicks appeared otherwise to have developed normally.

In addition one should note the extremely high mortality (80%) and low hatching ability (4%) among the insulin-treated compared with the control groups (29% and 12% for the saline treated embryos and 26% and 21% for the untreated embryos).

The relatively high mortality in the control groups can partly be ascribed to the lower quality and hatching ability of the eggs in the winter when the experiments were being carried out. This point has previously been made by *e.g.* Hutt & Greenwood (8). Some slight temperature variations in the incubator can also have contributed to the low hatching ability. The difference in this respect between saline treated and untreated embryos is probably due to the injury induced in association with the injection.

The lack of major pathological changes histologically does not quite agree with the findings of Anderson *et al.* (9) who found some morphological but no histochemical abnormalities in the growth cartilage of insulin-dwarfed chicks. In this investigation, however, only the hip cartilage from ten randomly chosen chicks was studied and it must be pointed out that it is unknown whether or not structural changes occur in the embryos in which dislocation of the hip is induced.

#### S U M M A R Y

Experiments were made to try and produce congenital dislocation of the hip by injecting insulin into the eggs of incubating chicks. A total of 1379 eggs were treated. Of these only 17 could be assessed with the methods used. They showed four clinically suspected dislocations of which two could be roentgenologically verified. Apart from these, a relatively high incidence of generalized skeletal deformities and a high mortality was found.

As controls 111 eggs were treated with physiological saline and 598 incubated without any treatment. No pathological changes of importance were found in the control series. All the specimens were examined by X-ray. All the insulin treated and untreated embryos as well as almost one half of the saline treated embryos were examined by the alizarin method.

Ten of each of the insulin treated and the untreated embryos were examined histologically, but no differences in cartilaginous and bony structure could be shown between the insulin treated material and the normal.

## RESUME

Des expériences ont été entreprises pour essayer de provoquer une dislocation congénitale de la hanche par injection d'insuline dans les oeufs d'incubation de poulets. 1379 oeufs au total ont subi le traitement. Parmi ceux-ci 17 cas seulement ont pu être constatés par les méthodes utilisées. Il s'agissait de quatre dislocations que l'on a pu soupçonner cliniquement et dont deux ont été vérifiées radiologiquement. A part ces cas, on a constaté une fréquence relativement élevée de déformités squelettiques généralisées et un taux de mortalité élevé.

A titre de contrôle, 111 oeufs ont été traités par sel physiologique et 598 incubés sans aucun traitement. Les modifications pathologiques trouvées dans les séries de contrôle étaient insignifiantes. Tous les spécimens ont été examinés aux rayons X. Tous les embryons traités par l'insuline ou non traités, ainsi que la moitié environ des embryons traités au sel ont été examinés par la méthode de l'alizarine.

10 des embryons traités par insuline et 10 embryons non traités ont été examinés histologiquement, mais aucune différence dans la structure cartilagineuse et osseuse n'a pu être observée entre les embryons soumis au traitement et les embryons normaux.

## ZUSAMMENFASSUNG

Versuche wurden ausgeführt mit der Absicht angeborene Hüftverrenkungen mittels Injektion von Insulin in die Eier ausbrütender Hühner zu erzeugen. Eine Gesamtzahl von 1379 Eiern wurden behandelt. Von diesen konnten nur 17 mit den verwendeten Methoden beurteilt werden. Sie zeigten vier klinisch verdächtige Verrenkungen von denen zwei röntgenologisch bestätigt werden konnten. Abgesehen von diesen wurde ein verhältnismässig häufiges Vorkommen von allgemeinen Skelettverbildung und eine hohe Sterblichkeit gefunden.

Zur Kontrolle wurden 111 Eier mit physiologischer Kochsalzlösung behandelt und 598 wurden ohne Behandlung ausgebrütet. Keinerlei pathologische Veränderungen von Bedeutung wurden in den Kontrollversuchen gefunden. Alle Präparate wurden röntgenologisch untersucht. Alle Insulin behandelten und nichtbehandelten Embryos, wie auch beinahe die Hälfte der mit Kochsalzlösung behandelten Embryonen wurden mittels der Alizarinmethode untersucht.

Zehn der Insulin behandelten wie auch der nichtbehandelten Embryonen wurden histologisch untersucht, aber keine Verschiedenheit

der Knorpel- oder Knochenstruktur zwischen dem Insulin behandelten und dem normalen Material konnte nachgewiesen werden.

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