the dogs. It appears that the main source of nervous energy concerned with the maintenance of the hypertension induced by section of the moderator nerves is derived from medullary cardiovascular centers. Our results suggest that the most important chronic inhibitory influence upon these centers is the moderator nerve system, for although certain autonomic zones of the cerebral cortex, hypothalamus, and the upper brain stem are capable of vasomotor effects when excited electrically, their elimination to a greater or lesser extent does not necessarily modify the activity of the medullary cardiovascular centers in dogs with neurogenic hypertension. In fact, the pressor and depressor responses elicited from these higher levels of the central nervous system are as marked in hypertensive as in normotensive animals(2). These observations have led us to the conclusion that the neurogenic function of cardiovascular "centers" situated cephalad to those in the medulla is directed toward a modulation of the existing cardiovascular status for temporary needs without regard for the prevailing blood pressure level or pulse rate. Thus our concept of the neurogenic control of the arterial tension is that the moderator nerve system acting at the medullary level is the most important homeostatic influence upon the blood pressure and that higher levels of the central nervous system produce essentially temporary modulating influences upon these vital centers. This does not deny that such temporary cardiovascular effects repeatedly induced might not result in a state of chronic hypertension in some indirect fashion such as by altering renal hemodynamics, but it seems to us unlikely that a purely neurogenic type of chronic hypertension would frequently exist without a disturbance of cardiovascular regulatory functions at the medullary level.

Summary. Chronic neurogenic hypertension in dogs induced by moderator nerve section producing an increase in the central excitatory state of the medullary cardiovascular centers, is not permanently influenced to a significant degree by lesions of the hypothalamus or its descending pathways compatible with survival of the animals. It is suggested that the most important neurogenic homeostatic influence upon the arterial tension is exerted by the moderator nerves at the medullary level and that higher levels of the central nervous system act as temporary modulating influences in response to momentary needs rather than as chronic adjusters of the arterial tension.

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In vitro Induction of Cartilage in Mouse Somite Mesoderm by Embryonic Spinal Cord. (20924)

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Evidence has been accumulating that the origin of axial cartilage in fish(1), amphibia (2), and the chick(3) is dependent upon association of somite mesoderm with spinal cord. An inductive influence of some kind between the 2 tissues has been postulated. Inductive tissue interaction in mammals recently has been described in simple 2-component systems *in vitro*(4), offering opportunity for analysis of mechanism(5). The present experiments were designed to determine whether inductive effects between spinal cord and somites could be demonstrated under similar conditions. For this purpose clusters of somites from 9-day mouse embryos were cultured in the presence or absence of

9-day mouse embryo spinal cord.

Materials and methods. All embryos were from the cross of highly inbred BALB/C females and C3H males. On the 9th day following the discovery of a vaginal plug females were killed by cervical dislocation and the embryos removed aseptically. From each embryo the transverse section containing somites 5 through 12 was dissected, and the region including the somites and the interjacent spinal cord isolated. This region was placed for several minutes in 3% trypsin in Tyrode's solution without calcium and magnesium salts(6). By drawing the tissue back and forth through a pipette orifice slightly smaller than the piece, accompanied by gentle manipulation with a knife, the somitic masses and the spinal cord could be separated from the loose mesenchyme with very little apparent damage to either. Somites in earlier stages of development (total somite counts below 20) separated individually and intact in the trypsin solution while the more advanced stages had to be cut apart. The latter may have included some mesoderm beyond the somite proper. Spinal cord pieces, at least largely free of mesoderm, were cut for culturing as complete lateral hemisections approximately 0.4 mm long. Tissues were cultured in Carrel D-3.5 flasks contaning a clot of 0.6 ml of chicken plasma and 0.8 ml of nutrient medium (horse serum, Tyrode's solution and 9-day chick embryo extract in 2:2:1 ratio). One milliliter of the nutrient was added as a supernatant which was changed daily. Tissues were oriented in a loose cluster in the clotting mixture at the glass-clot interface. Groups of 8 somites (numbers 5-12 inclusive) from the right or left sides of each embryo were cultured in separate clusters. Those from one side served as controls cultured without spinal cord, those from the other side were experimentals combined in a cluster with spinal cord. Direct comparison was made only between such paired cultures. Explants were in culture for 4 to 7 days, and were examined and recorded daily. Four pairs of cultures were fixed for sectioning, 3 on the fourth and one on the fifth day. The others were continued for 6 or 7 days and then implanted, by a procedure previously described(7), into the anterior chamber of the eye of adult males where they were left for an additional period of 8 to 20 days. In all, 57 cultures were prepared, 29 with spinal cord and 28 without. Cultures were fixed in Bouins (B-15) with 1% urea, eye implants in Zenker's with acetic acid. All tissues were serially sectioned at 10 microns and stained with hematoxylin and eosin. Embryos ranged in developmental stage from 12 to 26 somites. Somites from the earlier stages were simple block-like masses without evidence of regional differentiation into myotome, dermatome and sclerotome. Somites from the later stages, on the other hand, were distinctly differentiated, with dermatome and myotome distinguishable from the looser sclerotome.

Results. Culture behavior. By 24 hours in culture, somite clusters without spinal cord had fused and spread into a thin sheet. On continued culturing the sheet increased in diameter, becoming thinner and more transparent but remaining fairly cohesive with little peripheral migration of individual cells. All cultures of somites from embryos having fewer than 25 somites remained homogeneous (Fig. 1) throughout the culture period, with the exception of transitory formation of one or 2 tubule-like structures in a few cases. Two of 6 cultures of somites from embryos possessing 25 or 26 somites showed markedly different behavior. They were faster spreading, denser centrally, and formed cartilage rods and nodules in the denser area.

Somite clusters with spinal cord also showed rapid fusion, with some impression that the initial spreading of individual somites was faster in the direction of the spinal cord. After complete encirclement of the cord fragment, the tissue seemed thickest around the cord and thinned toward a border of peripheral, healthy fibroblast-like cells. Most cultures appeared heterogeneous centrally, and were larger in diameter than their simpler controls. Of 26 cases held 5 or more days in culture 23 formed cartilage rods and nodules in the thicker central area (Fig. 2). The 3 negative cases involved somites from embryos possessing 15-19 somites. Two of these formed cartilage or bone on subsequent implantation into the eye, the third did not.

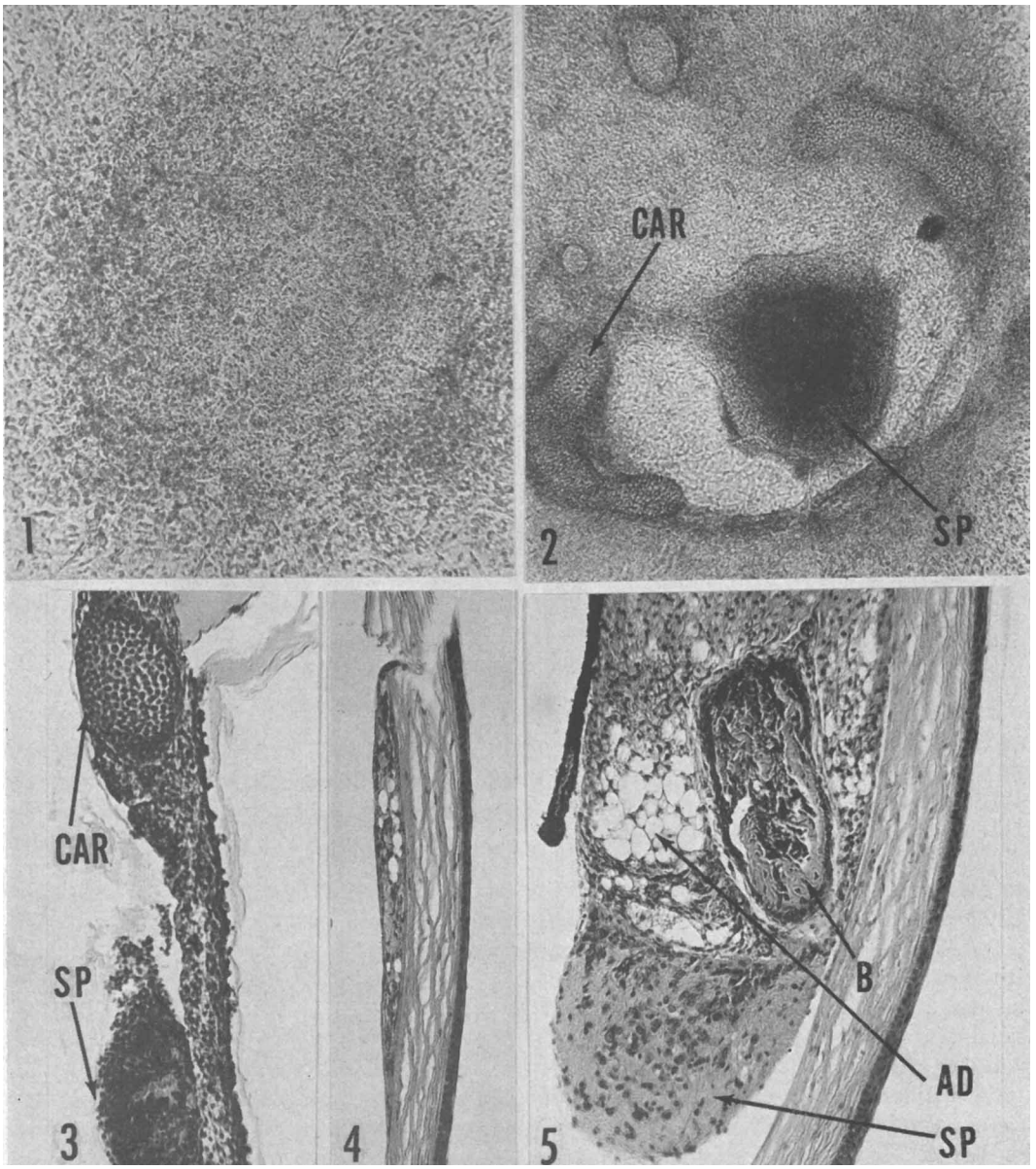

FIG. 1. Somites alone on 6th day in culture.
FIG. 2. Somites with spinal cord on 6th day in culture.
FIG. 3. As Fig. 2, stained section through cartilage nodule and edge of spinal cord tissue.
FIG. 4. Stained section of culture of somites alone after 18 days in anterior chamber of eye.
FIG. 5. Stained section of culture of somites and spinal cord after 18 days in anterior chamber of eye.
Magnification, Fig. 1, 2 = ×33; Fig. 3-5 = ×84. AD = adipose tissue; CAR = cartilage; SP = spinal cord.

Cartilage formation in culture usually was not immediately adjacent to the cord. This, together with the frequent conformance of the shape of the cartilage to that of the cord, was reminiscent of relations *in vivo*. However, these relations *in vitro* were notably variable, presumably in expression of tensional and other variations of culture conditions, and

their significance is not clear from the limited available data. In addition to the cartilage, small tubule-like structures appeared transitorily in several of the spinal cord cultures as in the controls.

Culture histology. Cross-sections of the clots fixed on the fourth and fifth days showed, in the absence of spinal cord, a thin layer generally not more than 1-3 cell layers thick, in which degenerating cells were present in varying frequency. There was little or no mitosis and no differentiation. In contrast, where spinal cord had been added the cultures were considerably thicker, especially in the vicinity of the spinal cord, and there was little evidence of cell degeneration except in the cord itself where healthy and degenerating areas were interspersed. Mitoses were discernible in the mesenchyme although not in particularly high frequency. The culture (24-somite stage) fixed on the fifth day showed well developed cartilage with surrounding perichondrium (Fig. 3).

Implant behavior. The thin, homogeneous cultures of somites alone underwent little or no growth when transplanted into the eye. With increasing time, the implants tended to become smaller until frequently only a thin diffuse remnant could be observed adhering to the cornea. Sections (Fig. 4) showed the implants to consist of a healthy but nondescript fibroid mass plus, where the source was embryos of somite number greater than 20, adipose tissue.* In 4 cases no trace of the implant could be found. The 2 exceptional cultures which showed heterogeneity and cartilage formation *in vitro* continued their exceptional behavior in the eye. These cultures of somites from 25 or 26-somite embryos formed healthy, vigorous masses which on sectioning were found to contain fibrous and adipose tissue as well as cartilage and bone. A third culture, from a 26-somite embryo, produced cartilage and bone in the eye although cartilage was not certainly identifiable during the *in vitro* period.

Cultures of somites combined with spinal cord, when implanted into the eye, characteristically rounded into a mass which became vascularized and grew vigorously. Opacities of varying size and shape could be discerned within the implants and, after the first week, adipose tissue appeared, frequently increasing to such an extent as to obscure other tissues. Sections of these implants (Fig. 5) showed either cartilage or bone, or both, in all but one case. Bone, well-differentiated with a surrounding periosteum and central hematopoietic tissue, usually had replaced most of the cartilage. Adipose tissue, with intermixed fibrous tissue, was conspicuous. In several cases large lymphoid masses were present.

Discussion. The results demonstrate that somites 5-12 of mouse embryos possessing a total of 12-24 somites do not form cartilage or bone when isolated in culture under the conditions specified, or upon subsequent implantation of the cultured tissue into the eye. Such somites combined with spinal cord, on the other hand, nearly invariably produce cartilage nodules in culture, and on subsequent implantation into the eye the cartilage is replaced in whole or in part by bone. Somites 5-12 without spinal cord, when taken from embryos beyond the 24-somite stage, do produce cartilage or bone in about half the cases in culture or on subsequent implantation, suggesting that at this later stage intrinsic tendencies toward differentiation of skeletal tissues have stabilized sufficiently to be manifested under these culture conditions.

The results give some support to the notion, based on studies of fish, amphibia, and the chick(1-3), that the spinal cord plays an inductive role in the formation of the axial skeleton. It must be noted, however, that the fact that spinal cord *can* induce cartilage formation in mouse somite material *in vitro*, while definitely suggestive, is not conclusive in demonstrating that it *does* function in this way in normal mammalian development. Caution is particularly indicated since a number of agents have been found to promote ectopic cartilage formation in adult mammals (8), and spinal cord is known to have not only

* Adipose tissue is here used to refer both to brown or hibernating fat and to white fat with typical signet-cells. Both types occurred in implants, occasionally as lobes of only one type, more usually as mixed masses with one type grading into the other.

tissue proliferative effects(9), but other morphogenetic effects which it clearly does not exert in normal development, *e.g.*, tubule induction in nephrogenic mesenchyme(5,10). The possible complexity of the spinal cord effect is suggested in the reported data, since the somite behavior is altered not only in its production of skeletal tissues, but in its much greater growth both in culture and in the eye, and in the formation of hematopoietic and occasional lymphoid tissue.

The mechanism of the spinal cord influence cannot be determined from available information. The usual appearance *in vitro* of cartilage at some distance from the cord fragment suggests that the influence may not depend upon direct contact between the interacting cellular masses. The possibility of a diffusible material, effective at some submaximal concentration and hence at a distance from the central source, has not received support from preliminary unpublished experiments involving interposition of a filter barrier through which effects of spinal cord on nephrogenic mesenchyme have been demonstrated(5). It will be of interest to determine whether the tubule-inducing and cartilage-inducing properties of embryonic spinal cord depend on the same or different mechanisms.

The frequent and conspicuous occurrence of adipose tissue and the absence of muscle in the grafts are of interest. Muscle might have been expected considering that the entire somite mass, including presumptive myotome, was explanted, and recalling that muscle was conspicuously present in intra-ocular grafts of similarly pre-cultured embryonic shields(11). That its absence is related to changes undergone by the somites while in culture is suggested by preliminary experiments involving shorter culture periods (unpublished data). Striated muscle has occurred abundantly, along with cartilage and bone, in implanted somite-spinal cord cultures of 24-hour duration. Similarly, evidence is accumulating that formation of adipose tissue is favored by longer periods of culture of nephrogenic mesenchyme, and this same mesenchyme can form striated muscle when combined with spinal cord in implants (unpublished data). Taken together, the data suggest that condensed mesenchyme from several embryonic sources can to a considerable degree be controlled in its subsequent differentiation *in vivo* by proper manipulation *in vitro*. The possibilities of analysis of the causal mechanisms in histogenesis of mammalian mesenchyme are thereby enhanced.

Summary. Somite mesoderm from the anterior region of mouse embryos possessing fewer than 25 somites fails to produce cartilage or bone either in culture or on subsequent implantation into the anterior chamber of the eye. When combined in culture with pieces of spinal cord from the same stage embryos, however, the mesoderm differentiates cartilage nodules in culture and produces bone including hematopoietic tissue, adipose tissue and occasional lymphoid tissue on implantation into the eye.

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