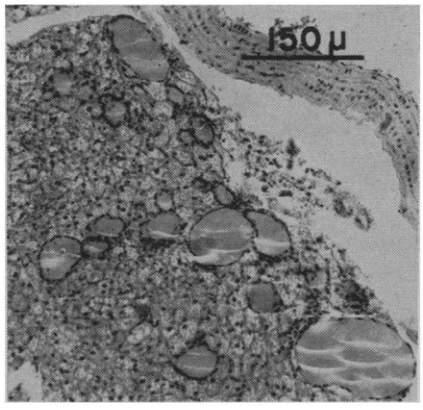

Fig. 1. A group of thyroid follicles within multilocular fat near, but not immediately adjacent to, the aorta. Hematoxylin and eosin stain (× 90).

floor of the pharynx to its normal cervical position, or from its descending beyond its normal adult location (*1*, *2*). In the first case aberrant thyroid tissue may occur at any median point from the tongue to its normal position and in the second case it occurs caudal to the normal position to include its location within the thoracic cavity. In man the thoracic thyroid has been described in the mediastinum behind the sternum, adjacent to the aorta, within the upper part of the pericardium, and in the interventricular septum of the heart (*2*). Another form of aberrant thyroid is the result of an anomaly of development of the fetal tissues as a whole. In man this is most common in the ovary and is considered a teratoma (*3*). Aberrant thyroid has been described in the rat (*4*), guinea pig (*5*, *6*), dog (*7*), rabbit (*6*), and baboon (*8*). I am not familiar with any reports of its occurrence in the mouse.

It is of importance to recognize the occurrence of aberrant thyroid tissue in experimental animals, for, as in man, unusual clinical pictures and thyroid function tests can result from the presence of aberrant tissue. In laboratory animals aberrant thyroid must be considered when unusual results are obtained from extirpation or other experimental procedures. Complete removal of the thyroid gland does not insure removal of all functional thyroid tissue.

Microscopic examination of tissues representative of all organ systems was conducted on 2634 mice of the BALB/c strain (without the milk factor) and 1033 mice of the Strong A strain. The heart of each mouse was serially sectioned from the apex to the base including the great vessels entering and leaving the heart. In seven BALB/c mice (five female, two male) aberrant thyroid tissue was observed at the base of the heart (Fig. 1). In six of these, the tissue occurred within the multilocular fat at the base of the heart near but not immediately adjacent to the aorta. In the other animal the tissue was within the pericardium at the base of the heart. In each of these mice a thyroid gland was present in the normal adult position. The aberrant tissue was identical in microscopic structure to normal thyroid tissue and in all probability was functional.

It is of interest that aberrant thyroid tissue was observed only in the BALB/c strain. In a comparison study on the normal thyroid gland of the two strains of mice, it was observed that in both strains the capsule is extremely thin or inapparent. In the Strong A mouse the thyroid gland is well confined and ends abruptly on all surfaces whereas in the BALB/c the thyroid is more loose and frequently strings out at the anterior and posterior poles of the gland. Small groups of follicles and occasional single follicles are frequently seen at either pole away from the body of the gland separated by multilocular fat and occasionally by striated muscle. This may indicate that portions of the thyroid anlage of the BALB/c mouse frequently do not descend with the entire structure or descend beyond the normal adult position.

Unfortunately, tissues were not removed from the pharynx or the base of the tongue, hence it could not be determined if lingual thyroid masses were present.

RONALD D. HUNT
U.S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado

References and Notes

1. B. J. Ficarra, *Diseases of the Thyroid and Parathyroid Glands* (Intercontinental, New York, 1958), pp. 211–222.
2. W. S. Rogers, in *The Thyroid*, S. C. Werner, Ed. (Hoeber-Harper, New York, 1955), pp. 283–242.
3. L. W. Sloan, *ibid.*, pp. 247–248.
4. J. H. Van Dyke, *Arch. Pathol.* **55**, 412 (1953); C. W. Asling, P. W. Durbin, M. W. Parrott, M. E. Johnston, J. G. Hamilton, *Proc. Soc. Exptl. Biol. Med.* **94**, 200 (1957).
5. C. D. Kochakian and D. Cockrell, *Endocrinology* **63**, 385 (1958); L. Spodafina, *Boll. Ist. Serioterap, Milan.* **8**, 619 (1929); H. Eitel and A. Loeser, *Arch. Exptl. Pathol. Pharmakol.* **167**, 381 (1932).
6. I. Leporati, *Boll. Soc. Med. Chir. Pavia* **51**, 1089 (1937).
7. R. G. Geil, *J. Am. Vet. Med. Assoc.* **138**, 539 (1961); M. C. Godwin, *Anat. Record* **66**, 233 (1936); P. Cohrs, *Berl. Tierärztl. Wochschr.* **46**, 683 (1930).
8. J. H. Van Dyke, *Arch. Pathol.* **54**, 248 (1952).

27 June 1963

Elasticity of Articular Cartilage: Effect of Ions and Viscous Solutions

Abstract. *The deformability of articular cartilage is increased by cations, more so by polyvalent than monovalent ones. Trivalent cations also depress elastic recovery. Failure of viscous solutions to alter the elastic behavior suggests ultrafiltration by cartilage as a possible mechanism in synovial lubrication.*

Deformability and resilience are major properties of articular cartilage that provide shock absorption and reduce friction in the functioning of joints. The elastic behavior of cartilage is, to a large extent, dependent upon mechanical extrusion and repenetration of tissue fluid, and is influenced by the concentration of salts in the milieu (*1*, *2*). In this study, the disarticulated heads of the tibias from 38 freshly killed dogs were used to test the effects of various ions and the viscosity of the immersion medium on the elasticity of the cartilage.

The cartilage was first equilibrated in physiological saline solution at 37 ± 1°C for 75 to 90 minutes. A compressometer (*1*, *3*) was then used to apply a static load of 83 g/mm² to the surface of the cartilage for 12 minutes. Linear deformation was recorded as a function of time, as was recovery when the load was removed. The measurements were repeated thereafter at the same site after the cartilage had again been equilibrated in the different solutions. The solutions, except where noted, were isotonic, calculated (*4*) as NaCl equivalents without correction for activity coefficients. The divalent cation solutions were 0.11*M* $CaCl_2$, $BaCl_2$, and $MgCl_2$; the trivalent solutions were 0.085*M* $AlCl_3$, $LaCl_3$, $FeCl_3$, and $[Co(NH_3)_6]$ Cl_3 (*5*). Monovalent cationic substitutes for Na^+ were 0.15*M* KCl, LiCl, and NH_4Cl. Tetramethyl ammonium chloride was used to provide a large cation that would be less firmly bound to the polyanions (*6*) of

the cartilage than the preceding cations. Monovalent (I^- and Br^-) and divalent ($SO^=$) salts of sodium were used for testing the effects of anions. Isotonic D-mannitol was used as a nonelectrolyte. Two- and 3-percent solutions of dextran (*7*) yielded viscosities of 21 to 62, relative to water at 37°C. Measurements were also made in bovine synovial fluid before and after treatment with testicular hyaluronidase ($\eta = 8.6$ and 1.2, respectively).

In the first test, the average deformation for 38 tibias, in 0.9-percent NaCl solution and after 12 minutes of loading, was 0.303 ± 0.013 mm. Twelve minutes later, the recovery was 98.3 ± 0.4 percent of the deformation. The mean ratio of the area under the curve of recovery to the area under the curve of deformation, as a function of time (Fig. 1), was 1.03 ± 0.01, because initially the elastic recovery was slightly more rapid than the deformation (*8*). Although the baseline deformations of the cartilage in 0.9-percent NaCl showed considerable differences between different animals, measurements made at the same site, on cartilage from the same animal, were readily reproducible. Changes in the extent of deformation and recovery in the test solutions are therefore presented (Table 1) as Δ(percent) of the original baseline deformation and recovery values in normal saline solution. Within each class of solute, the findings were consistent; for this reason the data were pooled.

The deformations in physiological salt solution approach, but do not usually reach, an asymptote between 12 and 18 minutes of loading (*1*). The shorter period was used in the present experiments for practical reasons. A few tests that were made over 18-minute periods gave results similar to those obtained at 12 minutes. These

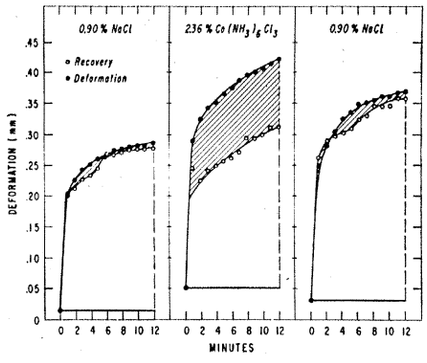

Fig. 1. Time curves of deformation and recovery in one experiment. Left to right: original deformation and recovery in normal saline solution; increased deformation and diminished recovery in cobalt; partial restoration of elastic properties in 0.9-percent NaCl.

observations, as well as the magnitude of the differences observed in the various groups of solutes, indicate that the principal changes in deformability represent changes in the "final saturation value" rather than simply changes in the rate of reaching it.

Removal of cations by distilled water or replacement by the $N(CH_3)_4^+$ resulted in smaller deformations of the cartilage without diminution of elastic recovery. The several monovalent cation solutions yielded data comparable to those obtained from cartilage in 0.9-percent NaCl. Conspicuous changes occurred in the solutions of polyvalent cations. Divalent cations caused marked increases in the extent of deformation of the cartilage with little elastic loss; the changes were largely reversed when the divalent cations were replaced by 0.9-percent NaCl. Trivalent cations caused shrinkage of the tissue, increased deformability, and caused a marked decrease in recoverability of the cartilage. Except with $[Co(NH_3)_6]^{3+}$, these effects were not reversible in physiological saline solution. The viscosity of the immersion medium did not influence the findings appreciably.

These observations may have a triple significance. The mechanism by which cartilage acquires its elastic properties has not yet been established conclusively (*9*). The protein-polysaccharide materials of the interfibrillar matrix, so far as is known, are viscous solutions rather than elastic gels. Nevertheless, the loss of rigidity of cartilage when its chondroitin sulfate is depleted by papain (*10*) indicates that the polysaccharide plays an important part. On the other hand, evidence has recently been presented that matrix polysaccharides do not contribute to the mechanical properties of rat tail tendon although the noncollagenous, interfibrillar proteins do (*11*). The present experiments show that anionic groups of the cartilage are involved in its physical properties; presumably the sulfate and carboxyl groups of the matrix polysaccharides and proteins are the principal anions concerned. The reduction in the electrostatic charge by the cations must, in some way, reduce the size of the colloid aggregates and thereby permit greater deformation and extrusion of water when the cartilage is compressed. Chondroitin sulfate binds counterions more like a polyelectrolyte solution than like an ion exchange resin, as has been suggested. The binding of counterions in such solutions becomes more pronounced when the cations have a higher valence (*9*), which is in accordance with the present findings. The relative reversibility of the cobalt-induced loss of elastic recovery of cartilage parallels the known competition between this cation and sodium for binding chondroitin sulfate. Whether the irreversible decrease in elastic recovery induced by the other trivalent ions is a salt effect or the result of protein denaturation of another type remains to be determined. The proteins of cartilage matrix presumably combine with cations. Certain trivalent metals also cross-link the collagen.

In speculations about the possible significance of cations in the pathogenesis of chondromalacia, the possibility that the presently unexplained degeneration of joint cartilage in hemophilia may result from presence of iron salts derived from the degradation of hemoglobin is attractive. Intra-articular injection of blood has been reported to produce degenerative changes in cartilage, although the specificity of the

Table 1. Changes in deformability and recoverability of cartilage in various solutions (mean ± standard error).

Original deformation in 0.9% NaCl (mm)	Second solution	No. of experiments	Δ Deformation (%)	Δ Recovery (%)
0.331 ± 0.042	0.9 % NaCl	5	0 ± 0.4	0.8 ± 0.3
.271 ± .062	3.4 % NaCl	3	27.3 ± 3.4*	−0.3 ± 1.1
.277 ± .028	H_2O, non-electrolytes	5	−17.3 ± 4.3*	0.2 ± 1.7†
.305 ± .042	$N(CH_3)_4Cl$	4	−8.3 ± 2.2*	−0.2 ± 2.1
.235 ± .004	Monovalent cations	5	4.2 ± 1.8	0.4 ± 1.6
.280 ± .003	Divalent cations	7	34.1 ± 4.4*	−1.3 ± 0.8
.365 ± .025	Trivalent cations	9	29.4 ± 7.8*	−34.1 ± 4.31*
.306 ± .025	Anions	4	2.4 ± 1.7	0.6 ± 0.4
.288 ± .065	Viscous, salts	3	1.5 ± 1.6	−1.9 ± 1.8
.383	Viscous, nonelectrolyte	1	−12.8	3.1

* $P < .01$ † Four experiments only.

lesion may be questioned. Trace metal content of synovial fluid has also been reported as being elevated in rheumatoid arthritis (*12*).

The prompt recovery of the cartilage following removal of load in the viscous media suggests that the tissue selectively excluded the long-chain polymers while allowing water and electrolytes to enter the tissue; that is, the tissue acts as a rapid ultrafilter. This may provide a mechanism for concentrating synovial mucin at the surface of the cartilage, thereby increasing the effectiveness of synovial fluid as a lubricant. It has been suggested (*13*) that the ground substance in vascular and certain other connective tissues may regulate ultrafiltration.

In an attempt to see whether the various ions caused swelling or shrinkage of the ground substance, paraffin-embedded sections were made of dog rib and rabbit ear cartilage that had been immersed for 2 hours in distilled water, isotonic Na^{+}, Ba^{++}, La^{3+}, and 3.4-percent NaCl. They were subsequently fixed in the same solutions to which formalin was added. No histologic changes in cells or matrix were detected in hematoxylin-eosin, periodic acid-Schiff, reticulum, or Rinehart preparations, with the exception of the chondrocytes which appeared less crenated following immersion in lanthanum than in the other solutions.

LEON SOKOLOFF
Laboratory of Experimental Pathology, National Institute of Arthritis and Metabolic Diseases, Bethesda 14, Maryland

References and Notes

1. S. M. Elmore, L. Sokoloff, G. Norris, P. Carmeci, *J. Appl. Physiol.* **18**, 393 (1963).
2. C. W. McCutchen, *Wear* **5**, 1 (1962).
3. Mr. George Norris designed a new flexible chamber for the compressometer to isolate the specimen and immersion solution from the temperature-regulating water bath.
4. J. M. Wells, *J. Am. Pharm. Assoc.* **5**, 99 (1944).
5. I am much indebted to Dr. Maxwell Schubert for the cobalt salt and for valuable suggestions; and to Mr. James F. Scott for technical assistance. The significance of ultrafiltration by cartilage as a possible factor in synovial lubrication was recognized during discussions with Mr. Frank C. Linn.
6. I. Kagawa and H. P. Gregor, *J. Polymer Sci.* **23**, 477 (1957).
7. Nutritional Biochemicals Corp., Cleveland; molecular weight, 5 to 40 $\times$ 10^6.
8. The curves in Fig. 1 are not typical in this respect.
9. D. Hamerman and M. Schubert, *Am. J. Med.* **33**, 555 (1962).
10. J. H. Bryant, I. G. Leder, D. Stetten, *Arch. Biochem. Biophys.* **76**, 122 (1958).
11. F. R. Partington and G. C. Wood, *Biochim. Biophys. Acta* **69**, 485 (1963).
12. W. Niedermeier, E. E. Creitz, H. L. Holley, *Arthritis Rheumat.* **5**, 439 (1962).
13. H. S. Bennett, *J. Histochem. Cytochem.* **11**, 14 (1963).

12 July 1963

Tanning in the Adult Fly: A New Function of Neurosecretion in the Brain

Abstract. *Tanning in the newly emerged fly is induced by a hormone secreted by neurosecretory cells situated in the pars intercerebralis of the brain. The same hormone is contained in the compound ganglion of the thorax, in concentrations six times as high as in the brain. This hormone is believed to act directly on the effector organ, and not through the secretion of ecdyson or a corpus allatum hormone; its release is effected through nervous impulses reaching the brain by way of the ventral nervous system a few minutes after the fly has emerged from the puparium. The hormone appears to be different from both the prothoracotropic and the gonadotropic hormones.*

In a previous communication (*1*) we described an endocrine mechanism which triggers off "tanning," the darkening and hardening of the adult fly after its emergence from the puparium. When the head is ligatured immediately after emergence the rest of the body never tans, but it can be induced to tan by injection of "active" blood from a 30- to 60-minute old fly. Simultaneous with the appearance of our report, a series of papers came out by Cottrell (*2*) dealing with exactly the same topic and with similar conclusions, but there were considerable differences in approach and details of technique. At the time of our earlier communication we had not succeeded in localizing the source of this hormone, but we concluded that it was not ecdyson, and did not originate from the corpus allatum or the brain. Similarly, Cottrell's experiments failed to identify the site of origin of this hormone. We now present evidence that, contrary to our earlier conclusions, the hormone is, indeed, a product of neurosecretion in the brain. Extracts from whole brains of newly emerged flies, which have inactive blood, or of 1-hour old flies, which have active blood, induced tanning in ligatured newly emerged flies, but extracts from the brains of flies aged 1 day or more were entirely inactive. There was little or no difference between the activity of brain extracts from newly emerged flies and 1-hour old flies. Extracts from the compound ganglion in the thorax were about six times more active than the brain extract from the same fly. The compound thoracic ganglion from older flies was entirely inactive. Extracts from the entire ganglionic system, including the brain, of feeding larvae, prepupae, untanned pupae, or 1-day old pupae, showed little or no activity, while extracts from the brain or thoracic ganglion from pre-emergent flies taken from the puparia were already active.

The hemolymph (blood) of newly emerged flies was already considerably active 2 to 3 minutes after emergence, as shown by a full tanning reaction in flies ligatured at that time. Fifteen minutes later the activity was so great that blood taken at that stage could be diluted up to 30 times with Ringer's saline and still cause full tanning when injected into ligatured flies. Evidently, at the height of activity in the blood, there is enough hormone present in one fly to induce tanning in over a hundred flies. Since the activity is so much greater in the thoracic compound ganglion than in the brain, it is assumed that most of the activity in the blood is derived from the thoracic ganglion. The disappearance of activity from the ganglion within a day indicates its release into the blood. This release must be triggered by an action of the brain, because it did not occur in the fly ligatured at the cervix.

The removal, at the time of emergence, of the median neurosecretory cells of the pars intercerebralis of the brain, by the technique of Thomsen (*3*), had the same effect as decapitation: tanning no longer took place. Extracts of brains from which the neurosecretory cells had been removed were entirely inactive. The activity of extracts made from the cells was about equal to that of an intact brain, although the section of the brain removed in the preparation of the extracts was probably less than one hundredth of the weight of the brain. However, implantation of intact cells had no effect at all, indicating that the activity is not released readily from the isolated cells. Corresponding experiments with the lateral neurosecretory cells indicated that they are probably not involved in this reaction.

In view of the much greater hormonal activity of extracts from the thoracic compound ganglion, and the fact that neurosecretory regions had never been described from this organ, we proceeded to search for such structures. Two groups of large cells which stain prominently with methylene blue