of this genotype were therefore examined at 2-day intervals from the time of eclosion to an age of 2 weeks. Arginine-rich histones were absent in males of all ages.

Table 1 shows that the defective histone transition is not a peculiarity of SD-72/SD(NH)-2. In all cases, sterile homozygotes produced late spermatid bundles lacking arginine-rich histones. Spermatid bundles from males of genotype SD(M)-35/SD-72 and SD(43)-1/SD-72 seem to have a slightly reduced amount of total histones as judged from the intensity with which bundles are stained; since the cytochemical assay is qualitative, not quantitative, this finding is at best only suggestive.

Two anomalies were encountered. Males of genotype SD(43)-1/SD(NH)-2 lack not only arginine-rich histones in their spermatid bundles, but also lysine-rich histones. However, the sterility of these males seems to be caused by a spermiogenic block that is unrelated to segregation distortion and which temporally precedes the block resulting from homozygosity for SD. Of 55 SD/SD genotypes examined by Hartl (*9*), SD(43)-1/SD(NH)-2 was the only one in which males never produced offspring. Other sterile SD homozygotes, including those in Table 1, produced a few offspring—less than 2 percent of the number produced by controls (*9*). Moreover, SD(43)-1/SD(NH)-2 males produce no motile sperm whereas all other SD homozygotes, even the sterile ones, produce large numbers of motile sperm (*9*). We therefore believe that the absence of lysine-rich histones in the late spermatid bundles of SD(43)-1/SD(NH)-2 males is not a result of homozygosity for SD.

The second anomaly in Table 1 concerns SD(Berea)-110/SD-72. In this case, arginine-rich histones were present in spermatid bundles. Since the fertility of males of this genotype had not previously been assessed, it seemed possible that such males might be fertile. Certain SD/SD genotypes are known to exhibit complementation at the *Sd* locus which leads to partial male fertility (*9*). Accordingly, males of genotype SD(Berea)-110/SD-72 were provided with groups of three virgin females at 2-day intervals for as long as the males produced offspring. Twenty SD(Berea)-110/SD-72 males produced an average of 222.8 ± 257.6 progeny per male; 19 control males produced an average of 1328.4 ± 249.5 progeny per male. The fertility of SD(Berea)-110/SD-72 males is therefore 17 percent of normal, a result which is well within the range of fertilities of complementing SD/SD genotypes (*9*). Thus, the presence of arginine-rich histones in late spermatid bundles of SD(Berea)-110/SD-72 males is accounted for by the large number of functional sperm that the males produce.

We conclude that sterile SD homozygotes have a defective transition from lysine-rich to arginine-rich histones during spermiogenesis. We must point out that the defective histone transition may be a secondary effect of homozygosity for SD; the primary lesion could be something quite different. Of interest in this connection would be an examination of individual spermatid heads in SD/SD$^+$ males. This is not possible with the cytochemical technique described here because the technique is not quantitative and because it is only sensitive enough to detect arginine-rich histones in entire spermatid bundles, not in individual spermatid heads. Nevertheless, our results strongly reinforce the suspicion that the developmental lesion in segregation distortion may be a defective transition from somatic histones to sperm histones.

Note added in proof: Ganetzky (*13*) has recently shown that *Sd* is probably between salivary chromosome bands 37D1-7 and 38B1-3, not near 39D-E, the locus of the somatic histones. Tokuyasu *et al.* (*14*) have studied spermiogenesis in heterozygous SD's with the electron microscope; the earliest observable abnormality presaging sperm dysfunction occurs in the chromatin in the latter half of nuclear condensation, corresponding in time with the histone transition. Evidently, *Sd* may be either a structural locus of sperm histones or a regulatory locus controlling the histone transition.

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1,25-Dihydroxyvitamin D in Biological Fluids: A Simplified and Sensitive Assay

Abstract. *A competitive binding assay for 1,25-dihydroxyvitamin D [1,25-$(OH_2D]$ in plasma has been developed in which intestinal cytosol preparations from rachitic chicks are used as the binding protein. A new method of extraction and two new chromatographic procedures are used for this assay. The method is sensitive to as little as 10 picograms of 1,25-$(OH)_2D$, and triplicate assays can be done on 5 milliliters of plasma. This assay shows that in the plasma of normal adult subjects there is a 1,25-$(OH)_2D$ concentration of 29 ± 2 picograms per milliliter, while none can be detected in the plasma of nephrectomized subjects and end-stage renal failure patients.*

Vitamin D requires hydroxylations in the 1 and 25 positions in order to express its biological activity in calcium, phosphate, and bone homeostasis. The resulting metabolite, 1,25-dihydroxyvitamin D [1,25-$(OH)_2D$], has marked antirachitic activity and is active in the stimulation of intestinal absorption of calcium and phosphate (*1, 2*) and of bone resorption (*3*). This metabolite results from the sequential hydroxylation of vitamin D on the C-25 in the liver and the C-1 in the kidney. The renal 1-hydroxylation seems to be the major site of regulation of vitamin D metabolism in response to the calcium and phosphate status of the animal (*4, 5*). Concentrations of the metabolites of vitamin D in the tissue and blood have been studied almost exclusively by administering isotopically labeled vitamin D compounds to vitamin D–deficient animals (*5*). Recently, however, Haussler and his co-workers (*6*) used a competitive binding assay to measure 1,25-$(OH)_2D_3$ in human and rat plasma. Their assay, for which they use rachitic chick-

en intestinal cytosol and chromatin, requires purification of the 1,25-$(OH)_2D_3$ in biological extracts through three chromatographic separations including liquid-liquid partition in order to remove interfering compounds.

We have developed an assay which is sensitive to 1,25-$(OH)_2D_3$ and to 1,25-$(OH)_2D_2$ in amounts as low as 10 pg. This assay includes a simplified extraction-purification scheme which recovers 68 percent of the vitamin D compounds while removing interfering compounds. The combination of sensitivity and high recovery means that the assay can be performed in triplicate on 5 ml of plasma.

The plasma extraction is performed in a separatory funnel. Plasma (5 ml) is placed in the funnel and 1,25-$(OH)_2$-[26,27-^{3}H]D_3 [70 pg; 3500 disintegrations per minute (dpm)] is added in 250 μl of 95 percent ethanol. Dichloromethane (methylene chloride; 30 ml) is added and the funnel is shaken vigorously for 5 minutes after venting. The dichloromethane layer is removed and the aqueous layer is reextracted twice more with shaking for 1 minute with 15 ml of dichloromethane. The pooled dichloromethane extracts are dried under reduced pressure and chromatographed on a 3.5-ml column (0.7 by 9 cm) of Sephadex LH-20 in a new solvent system: Skellysolve B, chloroform, and methanol (9 : 1 : 1). The entire extract is applied in 0.5 ml of solvent and rinsed with an additional 0.5 ml of solvent. The first 8 ml of elution solvent are discarded and the next 12 ml, which include the 1,25-$(OH)_2D$ are collected. This column fraction is dried in a stream of nitrogen gas and then subjected in toto to high pressure liquid chromatography on a Waters 10-μm silicic acid column (0.4 by 30 cm) at 800 psi (54.4 atm) with a flow rate of 1.8 ml/min and a solvent of 10 percent isopropanol in twice-distilled Skellysolve B. This solvent system (*7*) effectively separates all the known metabolites of vitamin D. The 1,25-$(OH)_2D_2$ and 1,25-$(OH)_2D_3$, which elute in the 20- to 23-ml fraction, are collected, dried in a stream of nitrogen, and redissolved in 95 percent ethanol. Two portions are taken for recovery estimation and three portions are set aside for assay. The overall recovery during extraction and chromatography is 68 ± 1.2 percent (mean ± S.E.; 20 samples).

White Leghorn chickens, which are raised from 1 day old on a vitamin D–deficient diet (*1*), are killed at 3 months. The duodenal loop is removed, rinsed with ice-cold phosphate buffer (0.05*M* potassium phosphate, *p*H 7.4, and 0.05*M* potassium chloride) and gently emptied. The mucosa is minced in five volumes of phosphate buffer and centrifuged at 700*g* for 10 minutes in the SS-34 R_{max} head of a Sorvall RC-5 refrigerated centrifuge at 4°C. This wash of the mucosa is repeated twice with fresh buffer and the mucosa is homogenized in two volumes of the phosphate buffer in a Potter-Elvehjem homogenizer. The homogenate is centrifuged at 226,660*g* for 40 minutes in the No. 50 titanium head of a Beckman L5-50 ultracentrifuge. The lipid coat is removed from the supernatant and discarded. The supernatant is lyophilized and stored at −20°C under nitrogen gas. The competitive binding assay is performed in polypropylene tubes (11 by 75 mm, Sarstedt) with standards in quadruplicate and samples in triplicate. By adding crystalline 1,25-$(OH)_2D_3$ in 50 μl of 95 percent ethanol to each tube, the standard curve is constructed from 10 to 100 pg. Seventy picograms (3500 dpm) of 1,25-$(OH)_2$[26,27-^{3}H]D_3 is added in 50 μl of 95 percent ethanol to each standard tube. A sufficient quantity of 1,25-$(OH)_2$[26,27-^{3}H]D_3 is added to each sample to bring the total content of 1,25-$(OH)_2$[26,27-^{3}H]D_3, including that which remains from the recovery estimate, to 3500 dpm. Samples with 0 and 5 ng of unlabeled 1,25-$(OH)_2D_3$ are treated in the same way so that the maximum and nonspecific binding, respectively, can be estimated.

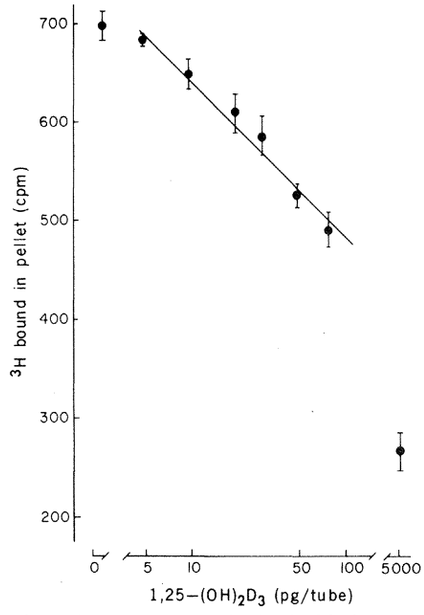

Fig. 1. Standard curve obtained with the competitive binding assay. Seventy picograms of 1,25-$(OH)_2D_3$[26,27-^{3}H]D_3 (9.3 c/mmole) prepared as described (*11*) was incubated for 1 hour at 25°C with 1 mg of intestinal cytosol protein and varying amounts of unlabeled 1,25-$(OH)_2D_3$. Protein-bound radioactivity was precipitated with polyethylene glycol (final concentration 20 percent, weight to volume). For each tube, the radioactivity (counts per minute) bound in the pellet is plotted against the amount of unlabeled 1,25-$(OH)_2D_3$ added per tube on a logarithmic scale. Maximum binding and nonspecific binding are determined in the presence of 0 and 5 ng of unlabeled 1,25-$(OH)_2D_3$, respectively, per tube. Points are the means (± standard error) of quadruplicate determinations.

Incubation is initiated by adding to each tube 1.0 mg of cytosol protein in 1 ml of phosphate buffer on ice. Samples are then gently shaken at 25°C for 1 hour and then replaced on ice. One milliliter of ice-cold 40 percent (weight to volume) polyethylene glycol (average molecular weight 6000 to 7500, Baker Chemical) is added to each tube, which is then vortexed and centrifuged at 9428*g* in the HS-4 head of a Sorvall RC-5 refrigerated centrifuge at 4°C for 1 hour. The supernatant is drained and the tip of the tube, containing the pellet, is cut off and counted in 4 ml of a *p*-dioxane based scintillation solution. This solution, containing 10 percent naphthalene and 0.5 percent 2,5-diphenyloxazole, provided 38 percent efficiency in a Beckman LS-100C scintillation counter at ambient temperature. The standard assay points of ^{3}H bound in the pellet plotted against the picograms of added unlabeled 1,25-$(OH)_2D_3$ on semilogarithmic graph paper yielded a straight line in the range of 10 to 100 pg of 1,25-$(OH)_2D_3$ per tube (Fig. 1). The standard curve is reproducible within 5 percent with different batches of intestinal cytosol. The 1,25-$(OH)_2D_3$ in the sample is calculated by reference to the standard curve of the ^{3}H bound in the pellet. This value is corrected for the percentage recovery previously determined for that sample. After such a correction has been made, the recovery of 1,25-$(OH)_2D_3$ added to rachitic chick serum is quantitative. The concentration of 1,25-$(OH)_2D_3$ in normal adult (*8*) human plasma was 29 ± 2 pg/ml (mean ± S.E.; 20 subjects). Plasma from five adults with advanced renal failure or nephrectomy (*9*) had no detectable 1,25-$(OH)_2D_3$. Plasma from four subjects aged 9 to 18 years had values ranging from 49 to 66 pg/ml (*10*). The reason for the apparent age dependency is being investigated.

The curves obtained with pure 1,25-$(OH)_2D_2$ were identical to those obtained with crystalline 1,25-$(OH)_2D_3$. Furthermore, the extraction and purification scheme was designed to recover the 1,25-dihydroxy derivatives of both vitamins D_2 and D_3. Hence this assay measures the 1,25-dihydroxy metabolites of both of these naturally occurring vitamins that are likely to be present in a normal human diet.

This assay has nearly twice the sensi-

tivity of the earlier assay (*6*). The amount of plasma 1,25-$(OH)_2D_3$ that is recovered during extraction and purification is one-third more than the amount recovered in previous methods, and the concentration of 1,25-$(OH)_2D_3$ in 5-ml samples of plasma can be estimated in triplicate. Because both 1,25-$(OH)_2D_2$ and 1,25-$(OH)_2D_3$ can be measured, a potential source of error is removed in estimations of 1,25-$(OH)_2D$ in the plasmas of persons on diets rich in either form of vitamin D. The concentrations of 1,25-$(OH)_2D_3$ and 1,25-$(OH)_2D_2$ that we found in the plasma of adults are somewhat lower than those found by Haussler and his coworkers (*6*), but this may reflect the age differences of the subjects. This assay is being used in investigations of several metabolic bone diseases.

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Modulation of Synaptic Transmitter Release by Repetitive Postsynaptic Action Potentials

Abstract. *The effect of repetitive action potentials in the postsynaptic axon on the release of synaptic transmitter from the presynaptic terminal was investigated at the squid giant synapse. Repetitive antidromic stimulation of the postsynaptic axon resulted in a reduction in the excitatory postsynaptic potential (EPSP). The reduction in transmitter release was accompanied by a decrease in the presynaptic spike afterhyperpolarization (AH). Increasing the concentration of extracellular potassium ions also reduced the EPSP and decreased the amplitude of the presynaptic spike AH. The reduction in transmitter release resulting from repetitive postsynaptic impulses is attributed to the accumulation of extracellular potassium ions. It is proposed that the accumulation of extracellular potassium ions resulting from repetitive postsynaptic activity may modulate synaptic transmission and function as an integrative mechanism in the nervous system.*

The modulation of synaptic transmitter release plays an important role in information processing in the nervous system and may be involved in the cellular mechanisms of learning and behavior (*1*). The mechanisms which have been reported to modulate synaptic transmission (*2*) include repetitive activity in the presynaptic terminals which can produce facilitation (*3*, *4*), depression (*4*, *5*), or posttetanic potentiation (*6*) of transmitter release. Other modulating effects

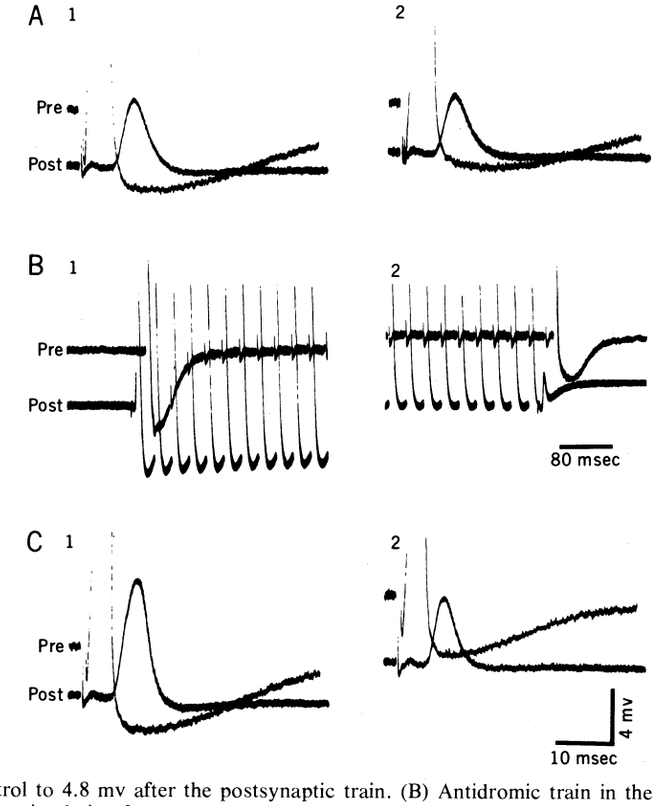

Fig. 1. Effect of postsynaptic stimulation and increased $[K]_o$ on synaptic transmission at the squid giant synapse. (A) The top trace is the record from an intracellular electrode in the presynpatic terminal showing the presynaptic spike AH. The bottom trace is the record from an intracellular electrode in the postsynaptic axon showing an EPSP. (A1) Control, recorded 10 seconds before the antidromic train in the postsynaptic axon shown in (B). (A2) Recorded 10 seconds after the antidromic postsynaptic train shown in (B). Note the reduction in the EPSP from 5.2 mv in the control (A1) to 4.2 mv after the postsynaptic train (A2). Note also the reduction in the presynaptic spike AH from 6 mv in the control to 4.8 mv after the postsynaptic train. (B) Antidromic train in the postsynaptic axon. The stimulation frequency was 40 hertz and the duration 20 seconds. This was recorded with standing spots on the oscilloscope and moving film; the spots were out of line such that the presynaptic record (top) is shifted to the right relative to the postsynaptic record (bottom). (B1) Beginning of an antidromic train in the postsynaptic axon (bottom trace). The top trace shows the AH of a presynaptic spike at the beginning of the postsynaptic train. The presynaptic axon was stimulated orthodromically every 10 seconds before, during, and after the postsynaptic train. (B2) End of the antidromic train of impulses in the postsynaptic axon. The top trace shows a presynaptic spike AH at the end of the postsynaptic train. Note the reduction in the postsynaptic spike AH from 5.2 mv at the beginning (B1) to 1.9 mv at the end (B2). Note also the reduction in the presynaptic spike AH from 6 mv at the beginning (B1) to 3.2 mv at the end (B2) of the postsynaptic train of impulses. (C) Effect of changing $[K]_o$. (C1) Control EPSP and presynaptic spike AH, as in (A1). (C2) Effect of increasing $[K]_o$ to 12 m*M*. Note the decrease in the EPSP from 8.6 mv (C1) to 4.9 mv (C2), and the decrease in the presynaptic spike AH from 6.4 mv (C1) to 4.6 mv (C2). All recordings (A, B, and C) were from the same synapse. The temperature was 7.0°C in (A) and (B) and 9.2°C in (C).