Reports

Sex Chromatin Anomalies in Newborn Babies in India

Abstract. The sex chromatin pattern in two series of newborn babies comprising 2058 males and 1832 females was studied to find the incidence of sex chromatin anomalies. We did not detect a single case of sex chromatin discordance when compared with the phenotypical sex. In this respect our survey differed from similar surveys in Canada, the United Kingdom, and Switzerland.

The first survey to detect sex chromatin anomalies in newborn babies was undertaken by Moore (*1*). Five of the 1911 males (0.26 percent) in his series showed the female sex chromatin pattern. The recent studies of Bergeman from Switzerland and Maclean *et al.* from the United Kingdom also found a comparable incidence (0.21 to 0.30 percent) of the sex chromatin discrepancy in phenotypical males (*2, 3*). The purpose of this communication is to report our findings in a similar survey carried out in Bombay, which suggests a very low incidence of sex chromatin discordance in males of our population.

Oral smears were collected from 3890 babies (2058 male and 1832 female) born alive at the Nowrosjee Wadia Maternity Hospital, Bombay. These samples were not collected from the consecutive births as in the aforementioned surveys, but were taken from two series of newborn infants. The first series comprised 1256 (669 male and 587 female) babies that were born during a specified time of every week, namely, 8 A.M. Monday to 8 A.M. Tuesday. The second series consisted of 2634 (1389 male and 1245 female) babies, and oral smears were collected from randomly selected cases. This random selection was according to the tables laid down by Fisher and Yates (*4*).

The smears were prepared by scraping the oral mucosa with a clean glass spatula and smearing on a small area of a glass slide previously smeared with Mayer's albumin. They were immediately fixed in Davidson's modified solution. The slides were stained by modified Feulgen technique (*5*).

From each smear 200 suitable cells were studied to calculate the percentage of the sex-chromatin body. The anatomical sex of the baby was unknown till the sex-chromatin pattern was recorded by two independent observers. Whenever the first smear was not satisfactory or where discrepancy was suggestive more smears were studied before the sex-chromatin pattern was finally recorded. For each case, a record of details such as anatomical sex of the newborn, mother's age, illness, if any, during the present pregnancy, and treatment, if any, was kept.

No discrepancy between the sex-chromatin pattern and the anatomical sex of the babies was found in our two series of cases. The sex chromatin count ranged from 0 to 3 percent among the male babies, while in the female it varied from 20 to 77 percent. The sex chromatin body, whenever present, was of normal size and shape.

The survey studies from Canada, Switzerland, and the United Kingdom have revealed that the incidence of sex chromatin discordance in the male at birth ranged from 0.21 to 0.30 percent. Assuming that a comparable incidence existed in our population we would have encountered at least four to six males showing the female sex-chromatin pattern. But as said before, our representative group of newborn babies—2058 males and 1832 females—did not reveal a single case of sex chromatin discordance. This may mean that a significantly lower incidence of this phenomenon occurs in the Indian population than that reported in the West. An alternative explanation—that a normal buccal smear in a phenotypical male is known to exist with sex chromosome mosaic, for example, XY/XXY, and that therefore we may have missed such cases in our series—is also compatible with our finding (*3; 6*).

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6. Grateful acknowledgment is made to Dr. K. M. Masani, principal medical officer of the Nowrosjee Wadia Maternity Hospital, Bombay, for permitting us to carry out this survey. Our thanks are also due to Dr. Lalitha, Dr. Kolah, and Mr. Nair for technical assistance.

1 May 1962

Freezing and Lyophilizing Alters the Structure of Bentonite Gels

Abstract. During quick-freezing with liquid nitrogen all but two molecular layers of water moved from between the crystals of saturated bentonite gels. The water molecules appear to have migrated to ice crystals forming near the clay crystals. Lyophilizing removed the ice crystals and further reduced interlayer water. Both quick-freezing and lyophilizing altered the original gel structure.

Recently lyophilization has become a common method of drying aqueous suspensions and gels of clays, particularly montmorillonitic clays. These clays shrink and form hard flakes when the gels are air-dried or oven-dried. However, the clay gels that are quick-frozen and dried by sublimation shrink very little, are soft and easy to powder, and seem to have largely retained the arrangement of particles that is thought

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

to prevail in the gel. This had led to the suggestion by Rosenqvist (*1*) that a lyophilized clay gel retains its original gel structure essentially unaltered.

If the gel structure could be retained unaltered during drying by lyophilization, then identical paths could be assumed for gas flow as well as for liquid flow through these clay plugs in fundamental flow studies. Other engineering properties, such as shear strength, could be studied under identical conditions with and without the liquid phase.

Recently Gonzalez and Vazquez (*2*) reported that lyophilization destroys the gel structure. They suggested that the structure is altered during the sublimation process and that the initial freezing does not affect the structure. Studies in our laboratory, however, show that both the initial quick-freezing and the sublimation process greatly alter the original gel structure.

Aqueous suspensions of bentonite (U.S. Pharmacopeia, particles less than 2 μ in diameter) were converted to calcium, sodium, or hydrogen-aluminum forms or left untreated. The gels were prepared by depositing the suspension onto porous ceramic tiles, removing the excess water by vacuum suction, and then equilibrating to 5 mm of water tension. Other samples were prepared by pouring thixotropic suspensions of bentonite into aluminum foil planchets.

The 001 spacings of the gels were measured on an x-ray diffractometer. The calcium, hydrogen-aluminum, and untreated bentonites all gave spacings of 20 to 22 A, indicating 10 to 12 A of interlayer water. The actual spacing of the sodium bentonite was not determined, but it expanded beyond 44 A.

The gels were then quick-frozen by being placed in liquid nitrogen. Diffraction patterns of the frozen gels showed, in all cases, that the 001 spacing of the montmorillonite-water system decreased to between 16 and 17 A. Norrish (*3*) reports that a monolayer of water on montmorillonites occupies about 3 A. The spacing of 16 to 17 A indicates an average of slightly over two layers of water in the interlayer positions. This indicates that freezing removes all but about the last two layers of water molecules from the interlayer positions of crystal groupings.

The manner of freezing appears irrelevant since slow freezing in a refrigerator or on a block of Dry Ice gave the same results. Upon the first appearances of thawing the samples reverted to their original gel spacings. Refreezing immediately decreased the spacings to between 16 and 17 A. Repeated cycles run on a hydrogen-aluminum clay showed that the process continued to be reversible.

Thus it appears that even the freezing which is associated with lyophilizing a sample greatly affects the original gel structure. It is postulated that the ice nuclei are formed in the more normal water in the larger pores between stacks of clay crystals, referred to as "domains" by Aylmore and Quirk (*4*), rather than between the clay crystals within a domain. A monolayer of water on each clay surface is apparently held by the crystal with energy greater than the energy that holds water molecules in ice. The movement of the water in and out of the interlayer spacings is evidently a very rapid process, since the freezing and thawing processes occurred in less than a minute.

Frozen calcium bentonite samples were lyophilized by placing the frozen samples in the lower part of a vacuum chamber which was then immersed in

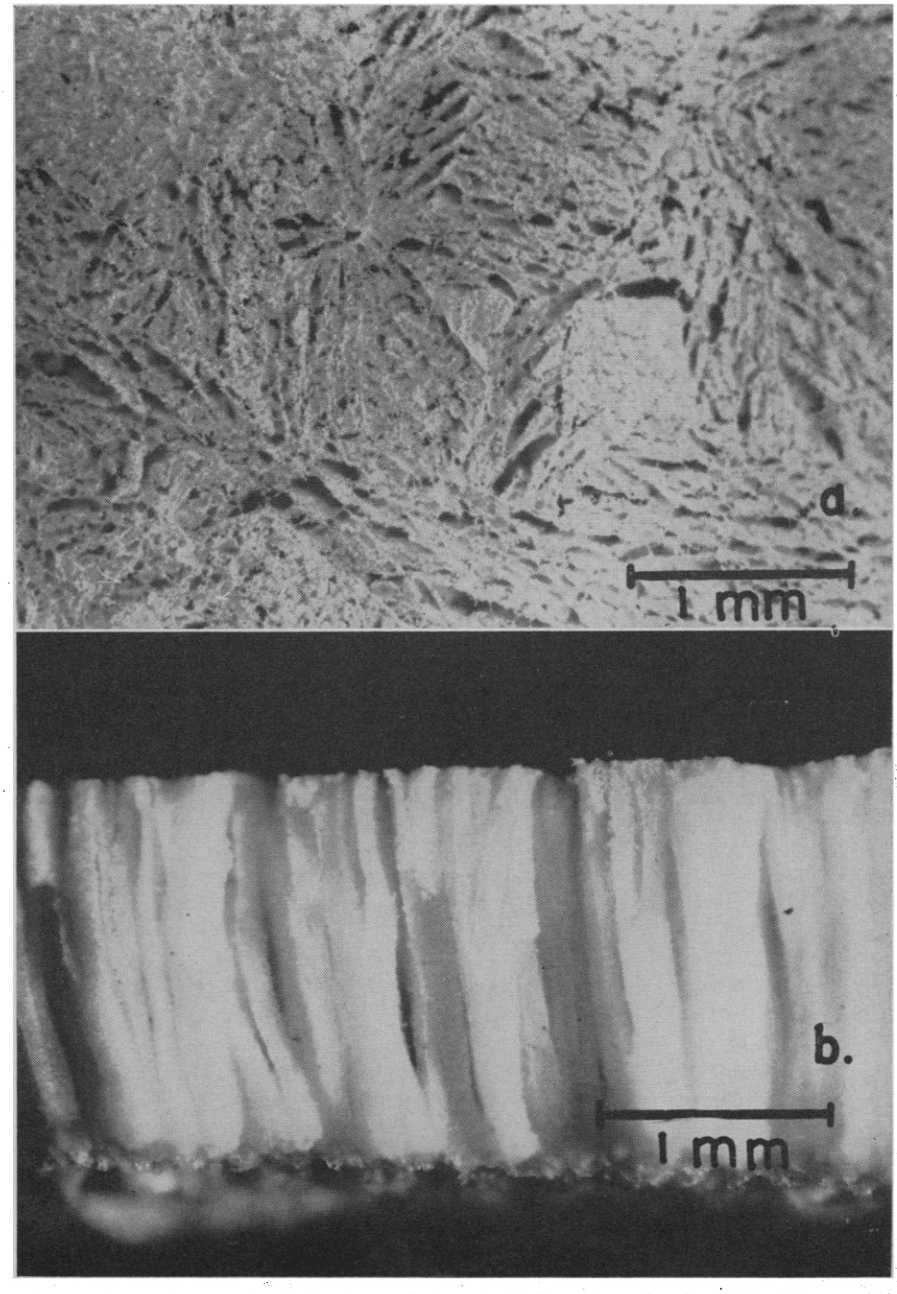

Fig. 1. Photomicrograph of (*a*) the surface and (*b*) the edge of a lyophilized bentonite gel.

a Dry Ice and methanol bath to keep the sample well frozen. The cold finger in the vacuum system contained liquid nitrogen. After the sample had been lyophilized to dryness the vacuum was replaced with dry nitrogen gas and the samples were stored for a moment over P_2O_5 until diffraction patterns had been run under a dry nitrogen atmosphere.

Although the lyophilized samples gave no visual evidence of shrinkage relative to the volume of wet gel, the diffraction patterns showed that the crystal 001 spacings decreased to 12 A. This is an even greater collapse than that caused by the freezing process, but it indicates that there is still a small amount of interlayer water since the crystals would collapse to a 10 A spacing upon oven drying.

Thus, x-ray diffraction studies indicate that either freezing or lyophilizing bentonite gels greatly alters their structure. Visual evidence of this was also obtained, by microscopic observation of the lyophilized samples (Fig. 1). Note particularly the fibrous nature of the gel in the edge view; subsequent studies indicate that this is due to the developmental pattern of the ice lenses. The dark areas in the photographs are voids left by the sublimed ice uptake.

Quick-freezing of thin (1 mm) samples of thixotropic bentonite gels on aluminum foil resulted in the freezing front's entering the sample from both surfaces at equal rates. The lyophilized specimens showed a parallel fibrous structure, similar to that shown in Fig. 1*b* except that the ice lenses entering from the two surfaces did not form all the way to the center of the sample. The center layer was without the fibrous structure. Thin gel samples froze more rapidly and developed a finer structure.

Word just received from Australia indicates that Norrish and Rausell-Colm (*5*) have found results similar to ours (*6*).

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27 March 1962

Classical Conditioning in Newborn Rats

Abstract. Newborn albino rats were trained according to classical conditioning procedure wih one of four intervals between conditioned and unconditioned stimuli. A vibrotactile stimulus (conditioned stimulus) paired with an electric shock (unconditioned stimulus) was presented to the forelimb 80 times. The results demonstrate that conditioning takes place in newborn rats. However, levels of performance as a function of time intervals between stimuli did not resemble the levels traditionally reported for older animals.

There is a paucity of experimental evidence demonstrating learning in newborn animals. In the experiment reported here we undertook to determine the ability of newborn rats to learn a simple conditioned response. The time intervals between presentation of conditioned and unconditioned stimuli were varied to determine whether the performance of the newborn rat is similar to that of older animals.

Newborn albino rats of the Sprague-Dawley strain (*1*) were tested on an apparatus for measuring delayed conditioned leg flexion. The animal was suspended in a harness to immobilize it in a nontraumatic manner. The conditioned stimulus was a vibrotactile stimulus delivered to the animal's chest by a glass rod attached to a speaker cone. The vibration of 128 cy/sec was produced by an audio oscillator set at an amplitude just sufficient to cause the rod to vibrate. No sound was emitted at these values. The unconditioned stimulus was delivered to the right foreleg by means of saline-saturated felt electrodes. This stimulus, of 50-msec duration, was a direct-current electric shock of 1.0 ma delivered through a current-regulating device which insured a constant current value regardless of the change in the animal's body resistance. All time intervals were controlled by electronic timers. Leg flexions were recorded by means of a microtorque motion-displacement transducer connected to the animal's leg by a fine thread. Spring tension on the transducer arm made it possible to record the leg movement. The output of this transducer, fed into an Offner (model A) electroencephalograph, was recorded, together with the time of presentation of the conditioned stimulus.

All animals received initial training with the conditioned stimulus only, within a period of from 1 to 8 hours after parturition, to insure that the stimulus would not elicit a leg-flexion response. The criterion for completion of this phase of training was ten consecutive presentations (conditioned stimulus only) without leg movement. Immediately after this, eight blocks of ten trials were presented, with random intertrial intervals of either 10, 15, 30, 45, or 50 seconds and an interblock interval of 3 minutes. Four groups of animals ($N = 9$ per group, randomly divided between males and females) were given paired conditioned and unconditioned stimuli on four different schedules, with intervals between the stimuli of 300, 600, 1200, and 2400 msec, respectively. For each experimental group a control group ($N = 4$ per group) was tested in a pseudoconditioning situation, with the same conditioned- and unconditioned-stimulus parameters, but with the order of the stimuli randomly varied.

The data presented in Fig. 1 demonstrate that newborn rats are capable of learning a simple conditioned response. Data for males and females within each of the experimental and

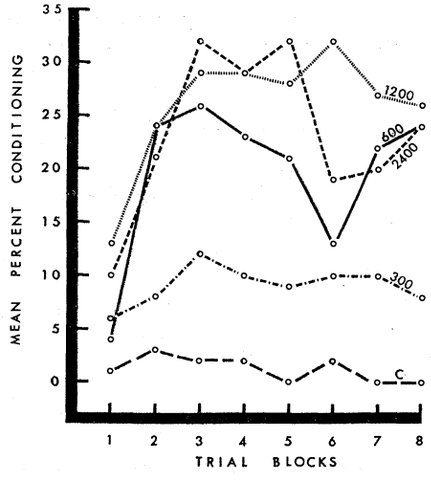

Fig. 1. Mean percentages of conditioned responses for newborn rats trained according to classical conditioning procedure with intervals between conditioned and unconditioned stimuli of 300, 600, 1200, and 2400 msec, respectively.