was then developed with acidic-ninhydrin reagent (*2*).

Under the conditions described, a separation of 5.5 cm has been obtained between glycine and trifluoroacetyl glycine, the acetylated derivative migrating to the anode and the glycine remaining almost stationary. The use of the same technique afforded a separation of 4.5 cm between alanine and trifluoroacetyl alanine.

It is conceivable that the technique described may be of use in evaluating (i) the hydrolysis of trifluoroacetyl amino acids by enzymes (*3*), (ii) the solubilizing effect of trifluoroacetic acid on proteins (*4*), and (iii) the cleavage of peptides after trifluoroacetylation of the peptide bond (*5*). In addition, the purity of trifluoroacetyl amino acids may be determined by separating large quantities of these compounds electrophoretically and then checking for ninhydrin-positive compounds (the free amino acids are ninhydrin-positive without basic hydrolysis) (*6*).

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26 September 1957

Occurrence of Plasmalogens in Lipides of Green Peas

The plasmalogens (*1*), a group of glycerophosphatides which contain a higher fatty aldehyde, have been reported to be constituents of various animal phosphatides (*2*). Recently Lovern (*3*) has shown that plasmalogens also occur in vegetable lipides. Commercial samples of the total phosphatide fractions of soybeans and peanuts possessed an aldehyde content equivalent to about 7 percent of palmitaldehyde. Plasmalogens are readily cleaved by acid and mercuric chloride, and the liberated aldehyde may be determined with fuchsin-sulfite solution (Feulgen reagent) (*4*).

The lipides of green peas (*Pisum sativum* L.) have been under investigation because of their probable role in the development of off-flavors during frozen storage of raw peas (*5*). Crude lipide material was extracted from lyophilized raw peas, Perfected Freezer variety, with chloroform-methanol, 2:1. The lipide components of the mixture were then separated by means of a solvent fractionation procedure (*6*). The various fractions were then analyzed for their plasmalogen content with Feulgen reagent. A reaction time of 4 hours at room temperature was employed. The color which developed was extracted from the aqueous reaction mixture with 4 ml of isoamyl alcohol and read at 572 mμ in a Beckman spectrophotometer, model DU, against reagent blanks. The amounts of plasmalogen were determined from a standard curve obtained with known amounts of palmitaldoxime treated as described above.

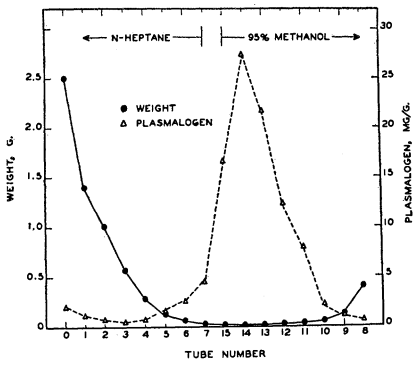

Fig. 1. Countercurrent distribution of acetone-soluble pea lipides between *n*-heptane and 95 percent methanol, showing distribution of weight (circles) and plasmalogens (triangles).

Crude pea lipides were separated into acetone-soluble and acetone-insoluble fractions, the latter primarily a mixture of phosphatides. Phosphatides may be classified on the basis of their solubility in glacial acetic acid and in 95 percent ethanol (*7*). Lecithin (phosphatidyl choline) and cephalin (phosphatidyl serine and phosphatidyl ethanolamine) are soluble in glacial acetic acid, whereas phosphatidyl inositol is not (*8*). Furthermore, lecithin is soluble in alcohol, and cephalin is not. The pea phosphatides (acetone insoluble lipides) were fractionated with glacial acetic acid and with 95 percent ethanol. The plasmalogen content of the phosphatide fractions (expressed as milligrams per gram of lipide) was as follows: alcohol soluble (lecithin), 3.9, alcohol insoluble (cephalin) 7.3, acetic acid insoluble (phosphatidyl inositol) 29.4. Thus the greatest concentration of plasmalogens would appear to be associated with the more complex inositol phosphatides which normally are insoluble in glacial acetic acid.

The acetone-soluble pea lipides were subjected to countercurrent distribution between *n*-heptane and 95 percent methanol in a seven-transfer system with single withdrawal (*6*) at a concentration of 13 percent with respect to each solvent (Fig. 1). It was anticipated that the bulk of the material would consist of triglycerides and that it would, therefore, be concentrated in the nonpolar solvent. In fact, over 75 percent of the total weight of lipides was found in the first three heptane fractions (tubes 0, 1, 2).

The plasmalogens were found to be distributed in the methanol fractions in the region of minimum weight distribution. The maximum amount was 27.5 mg/g in tube 14, which contained but 0.26 percent of the total weight. The tubes on either side contained lesser amounts of the plasmalogens, and only small amounts were present in the heptane fractions which comprised nearly all of the weight. Although phosphatides are normally considered to be insoluble in acetone, their presence in the acetone-soluble portion of a lipide mixture may be attributed to the well-known solubilizing powers of triglycerides.

Inasmuch as the separation of individual lipides from a naturally occurring mixture is always a difficult task, the apparent enrichment of plasmalogens by countercurrent distribution may provide a basis for the isolation and characterization of these unusual phosphatides in vegetable lipides. The lipides of green peas would appear to provide a convenient source of plant plasmalogens for such studies (*9*).

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23 September 1957

Sex Chromatin and Sex Differentiation in Human Embryos

In the past, embryologists studying sex differentiation in man paid little attention to the fact that *chromosomally* there exists no indifferent stage because every fertilized egg is either XY-male or XX-female. Chromosome analysis was

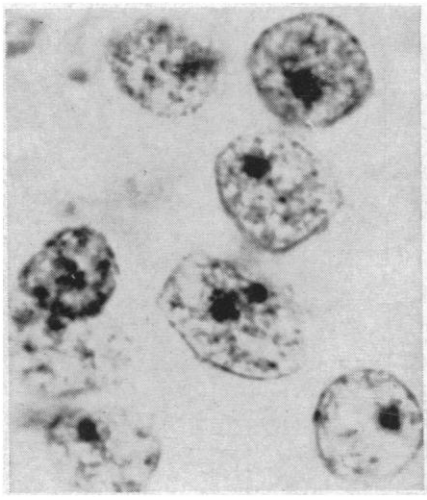

Fig. 1. Embryo, 2.4 mm (3 weeks). Heart mesenchyme, male chromatin pattern (× 1250) (hematoxylin and eosin).

too difficult for practical application. Now the situation has changed, since the Barr chromatin test (*1*) renders actual sex recognition possible even at earliest developmental stages (*2*). Recently, examining some embryologically indifferent stages in the Carnegie collection of human embryos, I found that nuclei of the heart mesenchyme are well suited for cytologic sexing (*3*). It is fortunate that a large proportion of the sectioned specimens of this collection are lightly stained in hematoxylin and eosin or in cochineal—techniques nearly as satisfactory as the more specific Feulgen method. Figures 1 and 2 show characteristic male and female chromatin patterns from embryos that had not yet developed any gonads.

In principle, the sex chromatin must be present in every female cell. However, not all tissues are favorable for its recognition. In the adrenal of a 140 mm female fetus it is not possible to point out the special nucleolus in the small, highly chromatic nuclei of the superficial cortex, while the deeper layers are favorable for its recognition (Fig. 3). Primordial germ cells, with their large vesicular nuclei, are also suitable material. Thus, the sex of migrating gonia may be recognized long before the organization of gonad primordia. In the cat, von Winiwarter and Sainmont, as early as 1909 (*4*), described a basophile nucleolus in prophase nuclei of ovogonia and ovocytes. They appraised it as a pair of sex chromosomes but were not aware that it might serve as a means of sex recognition.

Apparently the chromatin nucleolus is the product of allocyclic heterochromasy and conjugation of short segments (chromocentric blocks) of sex chromosomes. Electron-microscope studies indicate that, in the cat, the nucleolus contains four parallel threads. Most authors have assumed that the X chromosomes (carriers of female determining genes) are involved, but it seems equally probable that the chromocenters are parts of the Z chromosomes, which carry the male-determining genes. These questions are discussed more extensively elsewhere (*5*) and can be answered only by further investigations.

It is evident that embryologic sex differentiation can now be studied and evaluated with much more assurance. For some time it was realized that the divergence which histologically characterizes testicular and ovarian development starts somewhere in the seventh week—that is, in embryos of from 10 to 20 mm. In this range embryologists used to be reluctant to evaluate their observations and hurried on to the description of more advanced stages, of 24 mm and over. Now, with the chromatin test available, it is possible to make definite statements about the processes that initiate morphologic differentiation in man (*6*).

At 10 to 11 mm (stage 28), embryos have well-developed indifferent sex glands, with distinct cortical and medullary components. But even after the chromatin pattern has been ascertained, it is impossible to find a sexual difference in the histologic structure of the gonads. At stage 32 (17 mm), the gonads of both sexes have become distinguishable on the basis of structural changes near the surface. The primitive medulla is about equally well developed in both sexes, but the primitive cortex of male embryos begins to separate from the covering peritoneal epithelium, while in the female its adherence remains unchanged. Altogether, the ovary at this stage retains the appearance of the indifferent gonad, while in the male the entire subsurface layer of the primitive cortex becomes welded to the medulla. At the same time, seminal tubule formation becomes noticeable in this thick mantle layer, which contains the major-

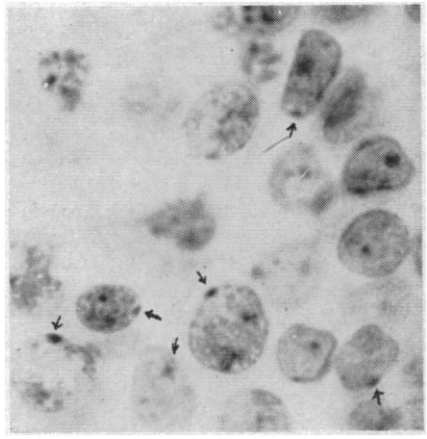

Fig. 2. Embryo, 3.7 mm (4 weeks). Heart mesenchyme, female chromatin pattern. Arrows point to chromatin nucleolus (× 1250) (hematoxylin and eosin).

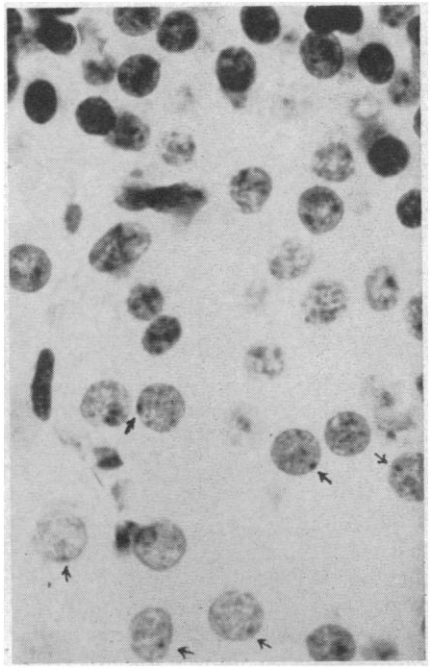

Fig. 3. Female fetus, 140 mm. Adrenal cortical tissue (× 1000) (Feulgen).

ity of all germ cells. The notion of there being first and second proliferations from a germinal epithelium is thereby rendered obsolete. It originated from misinterpretation of conditions observable in ovaries far beyond the stage of actual sex differentiation.

It will now also be possible to study, with some accuracy, the not at all rare intersexual traits of development at their earliest stages. In the course of his classical studies, Spaulding (*7*) came to the conclusion that the external genitalia of fetuses are distinctly male or female after the 25-mm stage. However, K. M. Wilson (*8*) showed that some of Spaulding's early "female" fetuses actually had testes. He declared that the appearance of the externals is an unreliable sex criterion before the stage of 50 mm. Reexamination of the pertinent slides in the Carnegie collection confirms the observations of Wilson.

But a more extended study raises two new questions. Up to what stage can a chromosomal male follow the female course and yet finally acquire normal male external genitalia? And which are the causes that delay, in some fetuses, the male differentiation of secondary sex characters? Both questions bear on the problem of male pseudohermaphroditism. Aberrations of this class show a full scale of manifestations, from so-called women with undescended testes to some minor degrees of hypospadias. Before 1950, even at the adult level, the chromosomal sex could only be guessed at. A case which once we assumed to be of female constitution (*9*) is now disclosed, by the chromatin test, to be a male (Fig. 4).

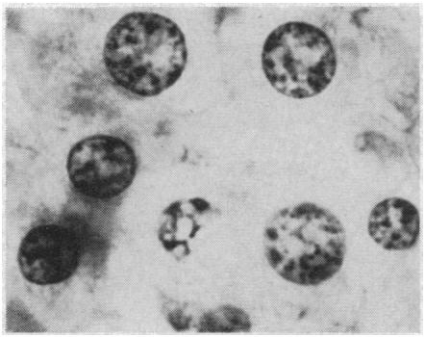

Fig. 4. Adult male pseudohermaphrodite. Interstitial cells of gonad, showing male chromatin pattern (× 1000) (Feulgen).

Obviously it is even more important to have the same guidance available in the study of aberrations at the embryonic stages. So far it appears that testis differentiation starts fairly regularly during the seventh week (about stage 30), but frequently it shows partial delays, with consequent persistence of cortical remnants and retardation of differentiation of the secondary sex organs. Transplacental interactions between male embryo and mother have been suggested as a possible cause (*10*), mainly on the basis of animal experiments (amphibian parabiosis). Recent developments, however, have opened direct approaches to this problem of human sex differentiation (*11*).

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23 September 1957

Operant Behavior during Sleep: a Measure of Depth of Sleep

Most animals spend approximately 30 percent of their lives asleep, yet remarkably few experimental investigations of sleep have been conducted, possibly because of the difficulty of measuring sleep. Processes that are difficult to measure may be studied in behavioral as well as in physical sciences by analyzing the frequency, duration, and degree of their interference with a more easily measured process (*1, 2*). In this report I present a method for measuring the duration and depth of sleep by recording how much it suppresses the rate of a reinforced operant response and compare the results with those obtained by measurement of body movements (*3*).

A sleep-deprived subject wearing an aviator's helmet was placed in a comfortable bed; the helmet contained an earphone through which a pure tone of 2000 cy/sec was delivered to the subject's ear. Each response (subject's thumb closing a microswitch taped into his preferred hand) was recorded on a counter and a Harvard cumulative recorder. A rate analyzer (*4*) controlled a potentiometer which reduced the intensity of the tone after each response. Rapid operation of the switch reduced the tone to zero intensity, and the subject could avoid the tone by continued responding. Slow operation of the switch kept the tone at a moderate intensity. If the switch was not operated, the tone rose to and was maintained at its full intensity (30 db). Thus the subject's rate of response controlled the intensity of the tone.

To record body movements, the base of a brass rod (9 in. long and ¼ in. in diameter) was suspended through the center of a brass washer (⅝ in. inside diameter) by a light spring from the center of the bed spring. A body movement was recorded when slight movements of the subject made the rod contact the washer.

Sleep records were taken under conditions of (i) 15 hours' sleep deprivation; (ii) 15 hours' deprivation plus 1½ grains of seconal ingested 5 minutes before retiring; (iii) 38 hours' deprivation; and (iv) 15 hours' deprivation without the tone. Since the latter condition was presented last, it provided a control for conditioned responding effects. Prior to the control session, the subjects were instructed to respond whenever they were awake at the rate they had on previous nights. Thus, behavior maintained by escaping the aversive tone could be compared with behavior maintained by recalled verbal instructions and previous conditioning. Two adult males, aged 20 and 34, served as subjects.

Figure 1 contains sample cumulative response records (selected as representative of 40 similar records) for one subject during eight continuous hours in bed on each of six different nights. Records for the first 4 hours (Fig. 1, top) show operant behavior during the deep initial

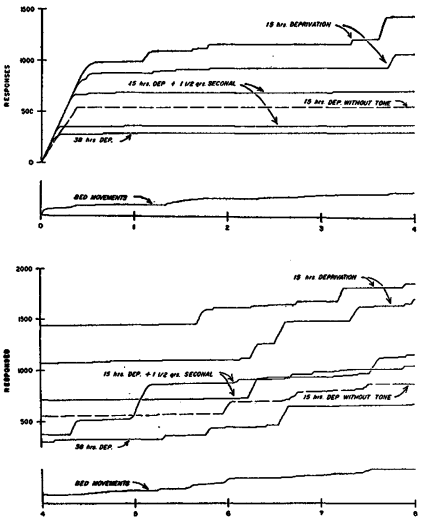

Fig. 1. Cumulative responses reinforced by a reduction in tone intensity are plotted against time in bed. (Top) First 4 hours in bed; (bottom) second 4 hours in bed. The lower the slope of the curves, the more intense was the tone and the deeper was the sleep. A cumulative record of body movements is presented at the bottom of each part.

sleep, and records for the last 4 hours (Fig. 1, bottom) show the subsequent light waking state characterized by bursts of responding. Records of short daytime naps contain response bursts very similar to those of the light waking state. The major effects of sleep deprivation and sedation on operant responding during sleep occur during the first 4 hours of sleep.

The two records for 15 hours' deprivation show the pattern of normal sleep. The subject spent 24 minutes in bed before the response rate dropped (sleep latency), and an additional 16 minutes passed before the rate dropped to zero (sleep onset). The period of deep sleep (from the time responses dropped to zero rate until 100 responses were emitted and during which the tone sounded at its full intensity) was 2 hours. Notable is the fact that approximately the same amount of responding occurred over the whole night on both 15-hour deprivation records, despite the separation of the two curves by 400 responses after 4 hours of sleep. The same effect appeared for the condition of 15 hours' deprivation plus seconal.

The addition of seconal to 15 hours' deprivation produced deep sleep sooner (sleep latency, 13 minutes) and more abruptly (sleep onset, 3 minutes) than did 15 hours' deprivation alone. Also, seconal doubled the deep sleep period (4 hours) and produced deeper sleep since fewer response bursts were emitted.

The 38-hour deprivation record was similar to the seconal record, with a short sleep latency (7 minutes) and an abrupt