separate mixtures of inorganic, ester, and nucleotide phosphates and to recover each of these in substance. The application of this information will, of necessity, be an individual one, and it seems not worth while to recommend a specific procedure of limited application. It must be kept in mind that differences in the degree of adsorption and desorption of esters and nucleotides as observed here will be encountered, and control experiments will be required (*7*).

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Exploration of Electronic Methods for Evaluating Sperm Motility

The growth of artificial insemination of cattle during the past 10 years has emphasized the need for adequate methods of predicting the fertility of semen samples before they are used. Laboratory studies and field operations have relied heavily on visual estimates of sperm numbers and sperm motility. These estimates have been continuously subject to the unavoidable human errors and biases encountered in collecting quantitative data with the microscope. A number of objective methods have been proposed for evaluating sperm motility (*1–3*). These methods have made use of the relationships among sperm numbers, area observed, distances traveled, and the time intervals between or during the observations. The objections to these methods have included such items as the need for standardization of the sperm numbers, the laborious and time-consuming techniques and calculations, the human bias, and the repeated high costs of the determinations.

The ideal method of evaluating sperm motility should include means of determining directly or indirectly (i) the total numbers of sperm per unit volume, (ii) the number of motile and the number of nonmotile sperm, (iii) the mean speed of motility displayed by the moving sperm, and (iv) the distribution of the total sperm with regard to kind and speed of motility.

A number of electronic methods have been proposed for making biological measurements (*2, 4*). Some of these methods have involved the quantitative measurement of some characteristic of a microscopic field by scanning techniques. Preliminary attempts at using such techniques for evaluating spermatozoa are reported here. Two principal assemblies of equipment have been utilized. The first of these was a model TA-124E Image Orthicon television camera chain, in which a type 58-20 Image Orthicon pick-up tube and a single-line selector were used to observe the oscillographic display resulting from single cells in the field. The second assembly consisted of a single, stationary multiplier phototube viewing a restricted portion of the microscopic field with the sperm cells rendered bright in a dark field either by dark-field illumination or by fluorescent staining and ultraviolet illumination (*5*). Impulses from the multiplier photocell were displayed oscillographically (with a triggered 1-second sweep and an a-c coupled vertical amplifier) and counted with a preset counter.

With the television camera chain assembly, the determination of the total numbers of cells in an optical field may be accomplished by relatively simple scanning techniques which will count the total particles in the field (*6*). Two fluorescent dyes, Coriphosphin and Euchrysin 2 at nontoxic levels varying from 1:1000 to 1:100,000, were used to stain the sperm and helped to eliminate the counting of particles of extraneous material such as fat globules in the milk used as a protective diluent (*7*). Exposure to the near ultraviolet light emitted by the high-pressure mercury vapor lamp (AH6) used had to be limited to avoid heat and radiation injury to the sperm.

The moving sperm could be detected by the single line selector as they crossed the single scan line. The counter was preset to record only the maximal impulses which were caused by the passing of the sperm head. An example of the oscillographic display caused by a fluorescent-stained sperm crossing the single scan line is shown in Fig. 1*A*.

With the previous knowledge on total numbers of sperm present and with the information obtained on the number of sperm that pass the scan line in a given interval, the average speed for all sperm of the sample (including nonmoving sperm) can be calculated. Added information on the percentage of sperm moving would be necessary to determine the average speed of the motile sperm. With the use of more complicated electronic equipment involving a guard spot or memory circuits, which would differentiate between counts of moving and nonmoving sperm, the same equipment could be adapted to determine the percentage of motile sperm.

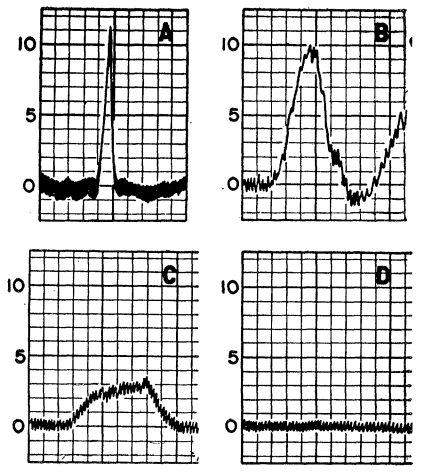

Fig. 1. *A,* Oscillographic display caused by fluorescent-stained sperm crossing a single scan line. *B, C, D,* Oscillographic patterns produced by dark-field illumination of sperm passing an aperture by swimming rapidly, swimming slowly, and by drifting, respectively.

A second attempt at evaluating sperm motility was made with the television camera chain by scanning an area of cell-free plasma into which sperm cells were allowed to migrate. The cells were either centrifuged to bring them down to an initial starting point or were allowed to migrate out of the opening of a stopcock into the plasma. The concentrated cells formed an opaque mass, and their migration into the plasma was easily followed by scanning the mid-section of the area with a single-line selector. The results obtained with this procedure were promising and suggested that refinements of the technique and the use of a lens system to magnify the field would add to the precision of the method.

When the secondary assembly of equipment was used, the multiplier phototube was placed so that it would pick up the image of a single sperm head as it passed an aperture. The impulse generated by the dark-field image of a sperm was counted in a manner similar to that used in the apparatus first described. In addition to counting the motile sperm which passed the aperture, it was possible to determine the kind of motility being displayed by a particular cell. Rapidly moving sperm which were exhibiting the normal helicoidal pattern of movement produced a sharp peak in the oscillographic sweep (Fig. 1*B*). A slowly rotating sperm produced a broad peaked sweep (Fig. 1*C*).

Oscillating sperm that were not rotating did not cause peaks in the horizontal sweep of the oscillograph (Fig. 1*D*).

Although the method in which the multiplier photocell was used gave the clearest recording of the type of sperm activity that was occurring, it did fail to give a measure of the total number of sperm present. This might be overcome by means of a mechanically operated stage which would permit counting the sperm in a known area of the slide by scanning with the multiplier phototube.

While none of the methods tried thus far have given results which approach the ideal for evaluating sperm motility, all the methods show considerable promise and are being investigated further. Relatively complex equipment was used for these tests as a matter of expediency. Simplified equipment to accomplish the same purposes is being assembled.

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Analysis of Small Amounts of Fatty Acids

A recent note by C. M. Coleman and G. Middlebrook (*1*) describes a new method for estimating small amounts of fatty acids. The authors claim that their method is the best described to date. The purpose of the present note is to draw the attention of the authors and of others working with fatty acids to a widely applicable general method published last year (*2*). In this method, as in that of Coleman and Middlebrook, ionic dyes of opposite charge to large organic ions are used in a two-phase system, consisting of water and an organic liquid. Unlike the method of Coleman and Middlebrook, in which use is made of the interfacial enrichment of the dye, this method makes use of the preferential partition of the stoichiometric simple salts that dyes form with large organic ions (*3*) into the organic phase. This makes the method an equilibrium method, in contrast to that of Coleman and Middlebrook. The method is, moreover, considerably more sensitive and accurate than that of Coleman and Middlebrook. For lauric acid, for example (a system for which their method did not give any result), estimation as the sodium salt of about 20 mμmole to approximately 1-percent accuracy can be carried out through the use of pinacyanol under proper conditions (*2*).

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19 August 1957

We wish to thank Mukerjee for having called his paper of 1956 to our attention. At the time of submission of our paper for publication in *Science*, reference to Mukerjee's work had not yet been indexed under a subject heading in *Chemical Abstracts*, and, consequently, we missed the original publication.

We have tried Mukerjee's method and agree with him that it is an excellent method not only for measuring small amounts of long-chain fatty acids but for measuring many other ionic surfactants. We would like to point out, however, that our method, in our experience, is more specific for the analysis of long-chain fatty acids in mammalian blood and tissue lipids. For example, phospholipids do not interfere with the determination of long-chain fatty acids in our method.

Our ignorance of Mukerjee's work very aptly points up the need for more prompt and efficient centralized transfer of information between investigators.

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An Enzymatic Basis for the Gluconeogenic Action of Hydrocortisone

The pronounced effects of cortisone and related steroids on carbohydrate metabolism appear to be due to their activity on hepatic gluconeogenesis rather than on the peripheral utilization of glucose. However, the biochemical mechanisms mediating the gluconeogenic action of these compounds are not known. Recently, Gavosto *et al.* (*1*) suggested that cortisone increases gluconeogenesis and imposes a negative nitrogen balance by enhancing transamination processes. They observed that near-toxic doses of cortisone (120 mg/kg), when administered to rats for 3 days, increased the activity of glutamic-oxalacetic transaminase (GOT) by 67 percent and that of glutamic-pyruvic transaminase (GPT) by 81 percent in liver. Independently, in the course of studies on the effect of hydrocortisone on enzymes which require pryidoxal phosphate as a cofactor, we have noted as much as 500 percent increase in the GPT activity in livers of rats treated with hydrocortisone, whereas, under the same conditions, the values for GOT were only slightly higher than those of the untreated control animals.

Male albino rats (Holtzman) weighing from 125 to 150 g were used. The methods of Lowry and co-workers for GOT (*2*), lactic acid dehydrogenase (*3*), and protein (*4*) were used. The method used for GPT (*5*) is based on the same principle as that of Wroblewski and LaDue (*6*), except that instead of measuring spectrophotometrically the disappearance of reduced diphosphopyridine nucleotide (DPNH), the DPN formed is determined fluorometrically (*3*). Tissues were kept at 0°C until they were homogenized and assayed. These enzymes were found to be stable during storage at −20°C in the 1 to 20 homogenates of brain and liver.

The marked rise in GPT activity, in contrast to the activity of GOT, in the liver of rats receiving hydrocortisone, is shown in Table 1. Lactic acid dehydrogenase (LDH), an enzyme which does not require pyridoxal phosphate, was not affected by treatment with hydrocortisone. Similar analyses of whole brain from the same animals revealed less than 20 percent increase in GPT activity and no significant changes in GOT and lactic acid dehydrogenase values associated with treatment. When pyridoxal phosphate was added to the complete system for each transaminase, no stimulatory effect could be detected. It was also noted that the addition of hydrocortisone in vitro to the GPT homogenate system did not enhance the activity of this transaminase.