denced by a prolongation of the duration of action. We would hope, in this way, also to accumulate naturally present GABA to threshold levels, which would then be recordable as synaptic inhibition.

On the basis that any alteration of GABA might be enough to terminate its inhibitory action, we attempted to interfere with its transamination with alpha-ketoglutaric acid. We have utilized *p*-benzoquinone, which is cited by Braunstein (*9*) as being a highly potent transaminase inhibitor against glutamic aminopherase, but we have been unable to alter the course of GABA effects in this way.

Meantime, because of the long-entertained possibility that events in the glutamic acid–gamma-aminobutyric acid equilibrium might play a role in altered excitability states of the brain and abnormalities—for example, in epilepsy—Woodbury (*10*) had examined the influence of diphenylhydantoin (dilantin) on GABA metabolism and had found in preliminary work that it increases the GABA content of cat brain. We therefore sought to prolong the GABA effects with dilantin. Figure 2 illustrates that we have succeeded in doing just this with intracarotid doses of dilantin having, per se, no effect on synaptic transmission. In fact, the prolongation starts only after a latent period, so that the effect of GABA introduced immediately after dilantin is not prolonged, but a test dose several minutes later does show a prolongation that persists in repeated trials over many minutes.

Perversion of GABA formation or destruction hypothetically constitutes one of the possible biochemical faults diagrammatically indicated at the top of Fig. 1, pointing out the potentially disturbed factors which might be the cause of synaptic dysequilibrium. If GABA is to be considered as a transmitter, then we may say further that it does not act at the same inhibitory receptors as serotonin, since we can effectively prevent or block the synaptic inhibitory action of serotonin with chlorpromazine but cannot similarly block that of GABA. Though not as potent as serotonin, GABA is of great interest because it is even more plentiful in the brain than serotonin and because its site of action is evidently not identical to that of serotonin (*11*).

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## Use of Charcoal to Separate Mixtures of Inorganic, Ester, and Nucleotide Phosphates

Several years ago, the use of charcoal for the separation of inorganic and nucleotide phosphorus fractions, with particular application to experiments involving radioactive phosphorus, was described (*1*). Briefly, the procedure called for the addition of charcoal to a trichloroacetic acid extract containing inorganic and nucleotide phosphorus, the removal by centrifugation of the charcoal with its adsorbed nucleotide, and heating of the charcoal suspended in acid solution to release the nucleotide phosphorus as inorganic phosphate. This procedure has been accepted and used routinely in a number of laboratories in spite of the limitation that the nucleotide is not recovered in substance.

In this laboratory, charcoal has been used principally for the removal of nucleotide from mixtures in which phosphate esters have been prepared by means of the hexokinase reaction (*2*). In the course of these experiments, it was found that the use of hydrochloric acid in place of trichloroacetic acid to terminate the reaction resulted in the loss of ester by its adsorption to the charcoal (*3*). Recent experiments on the adsorption of nucleotide by charcoal have led to the finding that desorption can be accomplished by suspension of the charcoal in trichloroacetic acid (TCA) to which has been added the ammonium salt of trichloroacetic acid. These observations are substantiated by the data of Table 1.

The experiments were carried out as follows: Five milliliters of a solution containing 1 μmole per milliliter each of inorganic phosphorus and of one of the phosphate esters shown in Table 1 were placed in a centrifuge tube. To this was added 0.05 ml of 5*N* hydrochloric acid and 0.3 g of acid-washed (*3*) Norit-A charcoal. The contents of the tube were mixed by inversion, 0.01 ml of 10 percent Triton X-100 (*4*) was layered on top to reduce the amount of charcoal which floats, and the tube was centrifuged. The supernatant solution was decanted and assayed for inorganic and total phosphorus by the method of Fiske and Subbarow (*5*). The charcoal was extracted twice with 5 ml portions of 0.05*N* hydrochloric acid by suspension and centrifugation and once with 4 ml of 10 percent trichloroacetic acid. The trichloroacetic acid extract was assayed for total phosphorus. The results of this experiment are given in the first two columns of Table 1.

A similar experiment was carried out with adenosine triphosphate and inosine triphosphate without the addition of inorganic phosphate. The charcoal, after the washing with 10 percent trichloroacetic acid, was suspended in 4 ml of 25 percent trichloroacetic acid, and a solution of ammonium trichloroacetate (prepared by the addition of 8.3 ml of 28 percent $NH_3$ to 20 ml of 100 percent trichloroacetic acid) was added to a final volume of 10 ml. The charcoal was mixed in by inversion and shaking and removed by filtration. The nucleotide in the various fractions was assayed by phosphorus analysis (*5*) of aliquots subjected to heating at 100°C in 1*N* sulfuric acid for 11 minutes (*6*). The results of this experiment are shown in Table 1.

These experiments show that it is possible, by the use of Norit-A charcoal, to

Table 1. The adsorption of inorganic, ester, and nucleotide phosphates by acid-washed Norit-A charcoal. TCA is an abbreviation for trichloroacetic acid. The concentrations of these solutions are given in the text. The percentage desorbed is calculated on the basis of the amount adsorbed, not on the amount in the original solution.

| Compound | Adsorbed from 0.05*N* HCl (%) | Desorbed in TCA (%) | Desorbed in TCA–$NH_4$TCA (%) |
|---|---|---|---|
| Inorganic ortho-P | 8.4 | | |
| Adenosine tri-P | 100 | < 1 | 91 |
| Inosine tri-P | 100 | 31 | 60 |
| Glucose-6-P | 91 | 97 | |
| Galactose-1-P | 96 | 100 | |
| Mannose-6-P | 93 | 88 | |
| Galactose-6-P | 91 | 92 | |
| Fructose-6-P | 92 | 100 | |
| 1,5-Sorbitan-6-P | 96 | 100 | |
| Fructose-1,6-diP | 97 | 95 | |
| Glucose-1-P | 97 | 81 | |
| Ribose-5-P | 100 | 75 | |
| L-Sorbose-1-P | 97 | 72 | |
| 2-Deoxyglucose-6-P | 100 | 53 | |
| Glucoheptulose-7-P | 77 | 83 | |

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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21 October 1957

## 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.

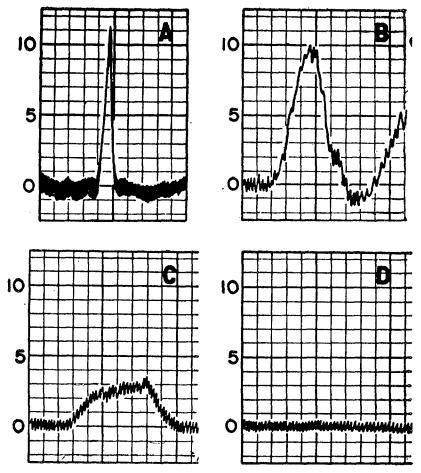


Fig. 1. *A,* Oscillographic display caused by fluorescent-stained sperm crossing a single scan line. *B, C, D,* Oscillographic patterns produced by dark-field illumination of sperm passing an aperture by swimming rapidly, swimming slowly, and by drifting, respectively.

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*).