Table 1. Sperm Density and Motility of the Semen Samples collected from Bulls fed 2 mg/kg Body-weight EDB Daily

Sperm density (cells per ml.)	Number of samples	Semen motility (% motile cells)	Number of samples
Up to 50×10^6	47	0–few	67
$50–100 \times 10^6$	18	5–10	6
$100–300 \times 10^6$	18	10–20	2
$300–450 \times 10^6$	5	20–40	5
Above 450×10^6	3		

The form of the cells was abnormal (tailless or with coiled tails, pyriform heads in various degrees of degeneration, Fig. 1).

In order to ascertain the reversibility of the treatment and the time needed for the EDB's action to be effective, EDB administration was discontinued on two of the four experimental bulls, renewed later to one of them and initiated in a third 16-month-old untreated bull of the same breed; this last-mentioned treatment was later stopped. In the first animal, when EDB had been discontinued for one month, almost normal spermatozoa (the acrosomic system did not seem entirely restored) were obtained; semen with normal density, motility and cell forms was obtained $3\frac{1}{2}$ months after EDB was discontinued. In the second bull, normal semen and spermatozoa were obtained 10 days after discontinuation of EDB. After two further weeks of administration, semen collected from this bull again began to exhibit the same abnormalities (Fig. 2). EDB was administered for 3 weeks to the third, previously untreated bull. Two weeks after the start of the treatment the semen of this bull began to exhibit abnormalities, which persisted for a month following the discontinuation of the treatment (Fig. 3). After that, the semen began to recover, but normal semen was obtained only after 2–3 months from the discontinuation date (Fig. 4).

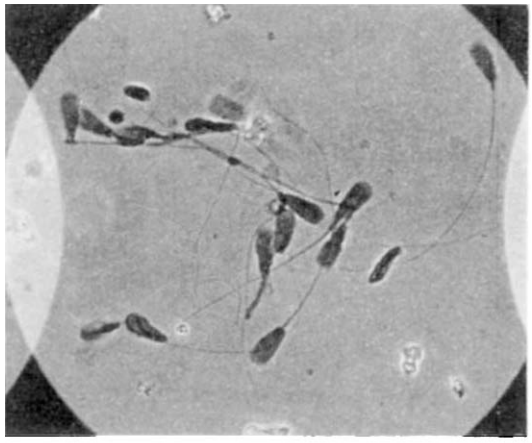

Fig. 1

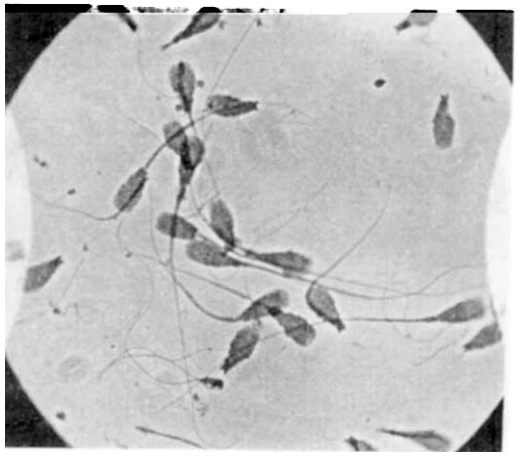

Fig. 2

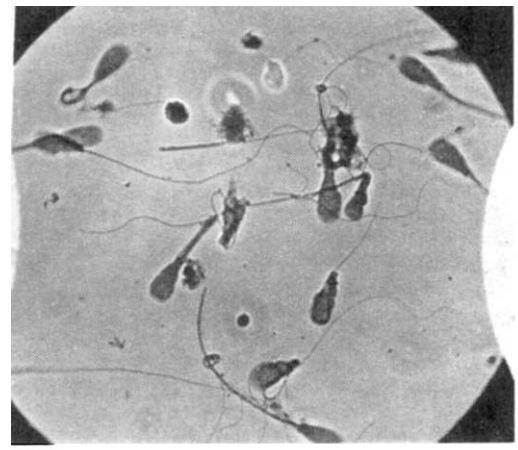

Fig. 3

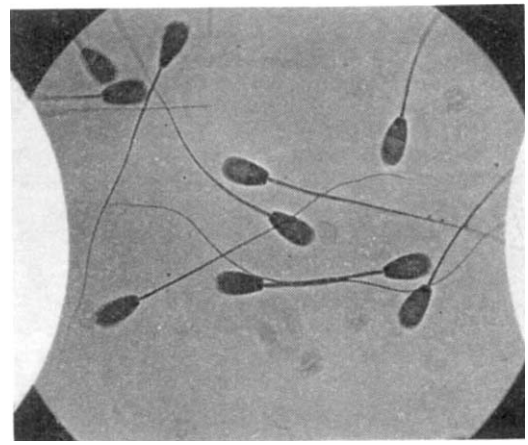

Fig. 4

From these observations it seems that, in response to a daily EDB dose of 2 mg/kg body-weight, bull semen density and motility decrease sharply, and the spermatozoa are of abnormal shape after two weeks of treatment. Recovery after discontinuation of the treatment varies from 10 days to about 3 months in different animals. EDB seems to affect the outer membrane and the acrosomic system of the spermatozoa. It is not clear whether the low sperm density is caused by complete degeneration of the spermatozoa or by the influence of EDB on the spermatogenic process.

This work forms part of a project supported by research grant *FG-Is* 117 of the U.S. Department of Agriculture.

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Simplified Method for Radioassay of Blood Glucose

Radioactivity in blood glucose following injection of ^{14}C-labelled precursor has been determined during numerous metabolic investigations. In most cases, either a comparatively large volume of sample[1] or a considerable amount of carrier glucose[2] has been used to prepare a

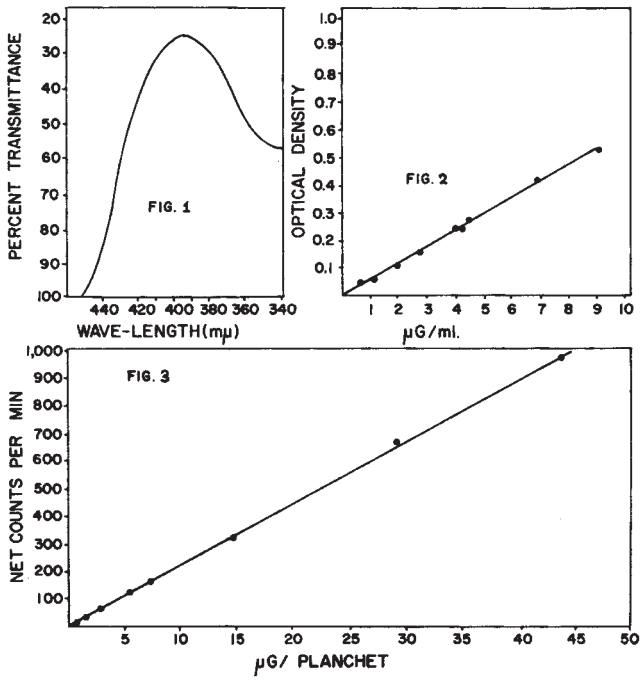

Fig. 1. Absorption spectrum of glucosazone solution

Fig. 2. Standard curve. Concentration of glucosazone versus optical density measured at 395 mμ wave-length

Fig. 3. Relationship between the number of counts per min and the quantity of glucosazone per planchet

sufficient quantity of glucosazone. Both these methods have disadvantages. Obtaining a large blood sample is nearly impossible in small animals and also has serious limitations on repeated sampling in large animals, while the addition of carrier glucose is limited to blood glucose which has a relatively high specific activity. Some investigators have counted glucosazone for radioassay at infinite thickness[3] while others have converted it to CO_2 and counted it as $BaCO_3$ at infinite thickness[1].

A simple method has been used in our laboratories which can easily be performed with as little as 1 c.c. of plasma, although a somewhat larger amount facilitates the preparation of glucosazone. This method does not require either the addition of carrier glucose or the conversion of glucosazone to $BaCO_3$. The overall method involves the preparation of glucosazone from plasma glucose, its colorimetric quantitation and counting for radioactivity.

Protein-free filtrate was prepared by Nelson–Somogyi's method[4] using $Ba(OH)_2$ and $ZnSO_4$ (10 ml. of each for 2 ml. of plasma). 20 mg of phenylhydrazine and 30 mg of sodium acetate were added to the filtrate. The solution was then heated for 3–4 h or until the volume was reduced to less than 2 ml. The osazone was allowed to crystallize overnight at 4° C and filtered through either a Coor's filterstick (*F*-3) fitted in plastic tubing or a planchet-maker with No. 2 filter paper disk. Osazone crystals were first washed twice with 2–3 ml. of water and finally with ethyl ether to remove excess phenylhydrazine and aniline. 5 ml. of 95 per cent ethanol were added to dissolve the remaining osazone. Recrystallization, if desired, could be done after reducing the volume to 2 ml. by evaporation.

Osazone was prepared from reagent grade D-glucose, recrystallized twice, and dried in vacuum to a constant weight. Standard solutions of various concentrations ranging from 0·7 to 36 μg/ml. were made in 95 per cent ethanol. An absorption spectrum (Fig. 1) revealed a single peak between a wave-length of 392–397 mμ. A standard curve was made by plotting the optical densities of the standard solutions measured at 395 mμ wave-length against their concentrations. Optical densities followed Beer's law up to the concentration of 9 μg/ml. (Fig. 2). The colour of the glucosazone solution deteriorated on prolonged storage. Osazone concentrations of the test solutions were calculated from the standard curve after determining their optical densities at a wave-length of 395 mμ using a 95 per cent ethanol blank. The glucosazone prepared from plasma had essentially the same absorption spectrum with a single peak between 390 and 395 mμ wave-length.

2 ml. of the test solution were applied to a stainless steel planchet, 3 cm wide, with four concentric rings, dried under a heat lamp and counted at infinite thinness in a gas-flow Geiger tube with an ultra-thin end window. No self-absorption was evident up to a quantity of 45 μg of osazone per planchet, as verified by counting different quantities of osazone made from radioactive glucose (Fig. 3).

The samples of radioactive glucosazone in 95 per cent ethanol were also counted with a liquid scintillation counter (Tri-Carb model 314 *EX*) using 15 ml. of a dioxane solution per 2 ml. sample. The results were essentially the same as those observed with the infinite thinness planchets although a higher counting efficiency was obtained. Because of severe quenching caused by the osazone, counting at infinite thinness was preferred.

This work was supported by U.S. Public Health Service grants *AM*–2396 and *2G*–633.

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PHARMACOLOGY

Degraded Carrageenan and Duodenal Ulceration in the Guinea-pig

Degraded carrageenan, given orally, will protect guinea-pigs from the peptic ulceration which can be caused by the injection of a suspension of histamine in a wax–oil base. The protection is seen principally in the duodenum where the ulceration is more severe in this type of experiment although some diminution of the gastric ulceration is also seen[1]. Duodenal ulceration is believed to be caused by excessive contact with abnormal quantities of highly acid gastric juice[2] and this has been accepted as true also of the experimental duodenal ulcer which follows histamine injection in the guinea-pig. Also, it has been shown recently[3] that histamine seriously disturbs the sulphate content of the mucosa and mesenchymal tissue of the duodenum and stomach of guinea-pigs, and eventually leads to a fall in sulphate content in the duodenum at the time and sites of formation of duodenal ulcer. Degraded carrageenan, a low-molecular-weight sulphated polysaccharide, has been shown to diminish the volume and acidity of histamine secretion[4], to interfere with peptic activity[5] and is believed to protect the mucosa[1] by complexing with mucoproteins so providing a protective coating more robust than mucin[1,6]. These facts constitute our knowledge of the mode of action of sulphated polysaccharides in experimental ulceration.

This communication extends this knowledge by showing that the anti-duodenal ulcer effect can be accom-