that is, giving approximately 50 per cent. crossing over between each lethal and "short." This shows more crossing-over than is known to occur in the X-chromosome of other species of *Drosophila* and may correspond to the greater length of the X-chromosome in *obscura*.

Further breeding tests showed the presence of two separate sex-linked lethals and agreed with the original assumption as to their loci. One (l_2) was found to be very close to the locus for "beaded" and gave 10 cross-overs with it in 155 males. "Beaded" was already known to be far enough from short to show no appreciable linkage to it. The other lethal (l_3) was independent of "beaded" but showed some linkage to "short" by a ratio of 21 "short" to 34 "not-short" males, while the number of "beaded" and "not-beaded" males was equal. That established its locus in the other end of the chromosome from (l_2) with "short" between.

33 (1493)

New methods for the analysis of cytoplasmic structures. With demonstrations.

By ROBERT H. BOWEN (by invitation).

[*From the Department of Zoölogy, Columbia University.*]

The year 1898 marked the recognition of two fundamental cytoplasmic structures,—mitochondria and the Golgi apparatus. The mitochondria soon became of interest to every biologist through Meves' theories concerning their importance in cellular differentiation and inheritance, but we are still very much in the dark regarding many features of their behavior. The Golgi apparatus, on the other hand, was almost forgotten and until recently even its status as an independent cytoplasmic element was in doubt. The last few years have witnessed in Europe a revival of interest in these and other cytoplasmic inclusions, an interest not yet fully reflected in this country. It would seem, then, an opportune time for calling the attention of biologists to these structures, so little reckoned within our physiological concept of the cell.

I have been experimenting with a variety of methods while studying the formation of the insect sperm, more particularly of Hemiptera belonging to the Family Cimicidæ (Pentatomidæ). Two new methods were found which have yielded interesting results.

The first is a modification of the Kopsch method for Golgi bodies, which depends on the reduction of osmic acid by the Golgi apparatus more quickly than by other cell constituents. This modification is more rapid than Kopsch and often gives good fixation of the cell as a whole. The procedure is as follows:

Fix in glass-stoppered bottles for 20 to 30 hours in Mann's sublimate-osmic:—

1 per cent osmic acid solution.....1 part
Corrosive sublimate, saturated solution in normal salt.....1 part

Wash in water over night, dehydrate and embed. Cut sections four micra thick. Mount directly or counterstain as desired; I find light green very useful. Golgi elements are intense black, mitochondria unstained or light brown. This method, in common with all Golgi technique, is more or less uncertain, due not so much to imperfect impregnation as to variability in general fixation. Since working with this method I have found that Weigl has made use of another sublimate modification for the Golgi apparatus.

The second method bears upon the structure of mitochondria, which, from a chemical standpoint, have been considered as a combination of some lipoid with an albumen. Further, it has been noticed, especially in Lepidoptera and Mollusca, that the mitochondria, occurring as spherical bodies, consist of an outer, intensely staining layer and an unstained medullary substance. Whether any correlation exists between these two facts is unknown. I have found that with Cajal's Golgi apparatus method[1] it is possible in the spermatid Nebenkern of these Hemiptera to demonstrate conclusively a compound structure, especially in those stages where the Nebenkern halves are elongating to form sheaths for the tail filament. Here the medullary substance impregnates intensely, while the chromophilic envelope remains

[1] For a readily accessible statement of this method see A. M. Pappenheimer, "The Golgi Apparatus," *Anat. Record*, 1916, Vol. 11, No. 4.

transparent. The medullary substance is at first arranged in a series of beaded threads running parallel to the long axis of the sheath, the threads undergoing a progressive side by side fusion, until eventually all merge into a single axial core for each of the now considerably elongated sheaths. This Cajal method, though notoriously capricious with the Golgi apparatus, gives some result for the present purpose in perhaps 75 per cent. of trials.

34 (1494)

The excretion of urea.

By J. H. AUSTIN, EDGAR STILLMAN and D. D. VAN SLYKE.

[*From the Hospital of The Rockefeller Institute for Medical Research, New York.*]

The excretion rate of urea in normal men increases directly as the blood urea concentration, and as the square root of the volume of urine, so long as the latter remains within ordinary limits, under about 5 liters per day. These relations are expressed in the formula, $D = KB\sqrt{V}$, D representing the rate of urea excretion, calculated as grams per 24 hours, B the blood urea concentration (grams per liter), V the rate of urine excretion, calculated as liters per 24 hours. When the size of the individual (W = weight in kilos) is introduced the equation becomes $\frac{D}{W} = KB\sqrt{\frac{V}{W}}$, or $K = \frac{D}{B\sqrt{VW}}$. $K = 7.5 \pm 3$ for normal individuals. When deficiency of the urea-excreting function is present K has a lower value. Increase in volume output beyond the rate of about 200 c.c. per hour, or 5 liters per 24 hours, ceases to accelerate urea excretion, so that for volumes of V in excess of 5, the figure 5 may be inserted in place of the actual V. The above formula gives more consistent results than that of Ambard, which assumes erroneously that the excretion rate increases as the square rather than the first power of the blood urea; and correlates the excretion with the concentration of the urea in the urine rather than with the volume of the urine. In calculating the rate of urine and urea output from short time periods, errors occasionally occur from