was inoculated at 60-day intervals with preserved material alone, and after 5 months all came down with primary tumors at the site of the last injection. This was followed by widespread tumor metastases, and death in all of the rabbits. Inasmuch as the preserved material which was used for the last injection had been refrigerated for 10 days instead of the usual 2 weeks, the possible presence of living cells capable of growth could not be ignored.

A definite enhancement, though somewhat lessened, has followed filtration of the preserved tumor material through "V" Berkefeld filters. The use of desiccated preserved material also results in a definite enhancement.

Animals of various age, sex, and breeds that have been tested so far have been suitable for inoculation with preserved material. A series of thoroughly tested and retested immune animals were inoculated with preserved material followed in 2 weeks by tumor inoculation. Thirty-three per cent of these immune rabbits grew malignant tumors; the others remained negative. Metastatic growth does not ordinarily occur from intracutaneous inoculation, but widespread metastases and death have resulted from the use of preserved material before skin inoculation.

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Some Vital Staining Reactions Bearing upon the Homology of Spermatocyte Dictyosomes.*

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In male germ cells the well-known dictyosomes and their derivatives, the acroblasts, are vigorously blackened by silver or osmium impregnation methods. Therefore they have been termed "Golgi bodies" and are accepted as complete homologues of the Golgi-apparatus of mammalian nerve and gland cells. The Golgi-apparatus is believed to be concerned with the function of secretion. An example frequently cited is that the acrosome of the animal sperm is secreted by the dictyosome complex, involving the tacit assumption that this complex is the homologue of the Golgi-apparatus. Recently this

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homology has been questioned. Parat[1] and his coworkers, for example, consider the dictyosomes to be chondriosomes of large and active type, partly for the reason that the dictyosomes (Parat's "lepidosomes") of the living cell stain with Janus green nearly as vigorously as the chondriosomes. Both the staining reaction and the implied homology have been doubted.[2, 3] It therefore seemed desirable to reinvestigate the problem.

The present account presents some results of intra-vitam staining of metamorphosing male germ cells of various insects. The species used were 2 Orthoptera of the family Acrididae, *Rhomaleum micropterum* and *Melanoplus femur-rubrum*, and 3 gryllid Orthoptera, *Oecanthus nigricornis*, *Nemobius fasciatus* and *Gryllus assimilis* var. *fluctuosus*. In the experiments with Janus green an hemipter, *Euschistus euschistoides*, was also used. Most of the work was done at the Marine Biological Laboratory, Woods Hole, Mass. The method used has been described previously.[4]

It is generally agreed that the mammalian Golgi-apparatus is not observed intra-vitam.[5, 6, 7] Several recent workers, however, have been able to see the dictyosomes clearly in unstained living spermatocytes. To the cases cited by Bowen[7] I have added the gryllid Orthoptera[4] and the insects listed above. In each of these the chondriosomes and the chromophilic part of the dictyosomes have almost the same high refringence and are easily identified in the unstained cell. Compared with the non-refringent Golgi-apparatus of the somatic cell, the dictyosome presents a physical difference which probably finds its basis in a dissimilar chemical structure. This assumption receives additional support from the vital or semi-vital staining reactions to be described.

Cowdry[8] has established that Janus green staining is the surest method for the demonstration of mitochondria (chondriosomes). It is agreed that in the mammalian somatic cell the Golgi-apparatus ordinarily is not stained with this vital dye (Gatenby,[9] Bowen,[7] Beams[5, 6]). However, when Janus green is applied to fresh smears

[1] Parat, M., *Arch. d'Anat. mic.*, 1928, **24**, 73.

[2] Hirschler, J., *Z. Zellf. mikr. Anat.*, 1928, **7**, 62.

[3] Gatenby, J. B., *Proc. Roy. Soc. London*, 1929, **104**, 302.

[4] Johnson, H. H., *Z. f. wiss. Zool.*, 1931, **140**, 115.

[5] Beams, H. W., *Anat. Rec.*, 1930, **45**, 137.

[6] Beams, H. W., *Anat. Rec.*, 1931, **49**, 309.

[7] Bowen, R. H., *Anat. Rec.*, 1928, **38**, 293.

[8] Cowdry, E. V., *Contrib. to Embryol.* (Carnegie Inst.) Washington, 1918, **8**, 39.

[9] Gatenby, J. B., Lee's Microtomist's Vade-mecum, 8th ed., P. Blakiston's Son & Co., Philadelphia, 1924.

of insect spermatocytes, vigorous coloration of chondriosomes and also the chromophilic part of each dictyosome results. In the spermatids the same is true; both the nebenkern and the acroblast complex are colored. This reaction has been obtained repeatedly in all of the insects studied, and a few tests gave the same result in Hemiptera of the family Reduviidae. These color reactions were obtained both with Gruebler's Janus green and with diazine green of National Aniline Co. Other mitochondrial dyes were used also. Methyl violet B (dahlia violet), while it kills the cell quickly, colors vigorously both chondriosomes and the crescentic, chromophilic rims of the dictyosomes.

In preliminary experiments with benzidine dyes, which ordinarily are not considered to be mitochondrial stains (see Gatenby[9]), similar results were obtained. The dyes used were trypan blue, trypan red, and pyrrol (isamine) blue. They tint both the chondriosomes and the chromophilic portions of dictyosomes to the exclusion of other parts of the cell, except the cytoplasmic chromatoid bodies (not the chromosomes). The color is rather delicate; the stains are not so vigorous as Janus green.

I have also used brilliant cresyl blue, which stains the chromophilic rims of the dictyosomes (and acroblasts) selectively, although prolonged application colors the chondriosomes lightly. Both are colored prior to the appearance of the well-known cresyl blue vacuoles, which I believe to be degeneration vacuoles. After slight application, the dictyosomes and cytoplasmic chromatoid bodies alone are colored. Fauré-Fremiet[10] and Karpova[11] were not able to stain dictyosomes with brilliant cresyl blue, but Avel[12] obtained a color reaction on the dictyosomes of the snail with it. The bodies of Perroncito in snail spermatids, likewise colorable with this dye, have been identified by Tuzet[13] as fragments of the acroblast remnant. Apparently the converse result is obtained with vital neutral fuchsine; my preliminary tests indicate that with it the chondriosomes alone are stained.

In a former work[4] I adduced evidence that chondriosomes and the chromophilic rim of the dictyosomes are chemically allied, namely: 1, both react positively to Sudan III; 2, they are preserved, stained or impregnated (as the case may be) in the same substances, differing only in degree; 3, both are stained vitally with Janus green.

[10] Fauré-Fremiet, E., *Arch. d'Anat. mic.*, 1910, **11**, 457.

[11] Karpova, L., *Z. f. Zellf., u. mikr. Anat.*, 1925, **5**.

[12] Avel, M., *C. R. Soc. Biol., Paris*, 1925, **93**, 161.

[13] Tuzet, O., *Arch. de Zool. exp. et gén.*, 1930, **70**, 62.

The present paper confirms and extends the third point, and adds a fourth, viz., confirmatory results obtained with benzidine dyes. If the dictyosome of the male germ cell is to be considered a homologue of the somatic Golgi-apparatus, the former must be composed in part of, or perhaps be invested with, a substance not present in or on the Golgi-apparatus. This substance, apparently much like chondriosomal substance, is refringent (probably lipoidal) in nature, causing the dictyosome to be visible even in the absence of stain. The material is perhaps present in widely divergent animal types. It is especially apparent in dictyosomes of gastropod Mollusca, well known for their marked refringence and susceptibility to vital staining, as demonstrated by Gatenby,[14] Avel,[12] and Karpova.[11] In prosobranchs, however, Tuzet[13] failed to stain the dictyosomes with Janus green, but she attaches little importance to the Janus green reaction, since she had formerly obtained a positive result in *Tubularia.*

In common with the post-vital reactions,[4] the results obtained with methyl violet, Janus green and benzidine dyes offer no obstacle to Parat's claim that the chromophilic part of the dictyosome is merely an hypertrophied chondriosome. That such conclusion is perhaps premature is indicated by the specific results obtained with brilliant cresyl blue (selective for dictyosomes) and with neutral fuchsine (selective for chondriosomes), but the value of these reactions for purposes of analysis may be, in some measure, open to question.

This study indicates that the complete homology of the dictyosome-acroblast complex of insect spermatogenesis to the mammalian somatic Golgi-apparatus remains to be established beyond a doubt, while the same might be said with equal justification of its affinities with the chondriosomes. Meanwhile the possibility remains that the complex in question is unique and peculiar to germ cells.

[14] Gatenby, J. B., *Quart. J. Mic. Sci.*, 1920, **64**.