THE GOLGI COMPLEX IN LIVING OSTEOBLASTS

GEORGE G. ROSE, M.D.

From the Department of Biology, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston; The Tissue Culture Laboratory, Hermann Hospital, Houston

ABSTRACT

Embryo chick leg bones were cultivated under sheets of dialysis cellophane in multipurpose culture chambers. The osteoblasts emigrating in this environment contained phase gray juxtanuclear bodies from which phase white droplets emanated. These were concluded to be the Golgi complex and are described with respect to their living morphology, microscopically observed functional activity, vital staining, and cytochemical reactions.

INTRODUCTION

Commenting on an analysis of Sjöstrand and Hanzon (1) which indicated that the Golgi apparatus did not undergo a structural change during the secretion cycle, Brachet (2) recently stated that it was rather unlikely that this organoid was directly involved in secretion. He did concede, however, that the evidence of electron microscopists indicated the existence of the Golgi complex, but that its role remained as enigmatic as ever.

The purpose of this report is to show by photographic illustrations topographic changes occurring in the Golgi complex of living cells in tissue culture during secretory cycles. This activity was observed in very prominent phase dark juxtanuclear bodies of emigrating embryo chick osteoblasts (3, 4) and recorded by time-lapse cinephase methods. Their form was quite similar to that observed in rat epididymal cells with phase contrast by Dalton and Felix (5) and Dalton (6), and proved by them with the electron microscope to be the Golgi complex. Although EM analyses were not used to corroborate the identification of the Golgi complex described in this report, morphological, physiological, cytochemical, and vital staining data are presented to conclude its identification and to establish its secretory function as a cyclic emanation of droplets into the cytoplasm.

MATERIALS AND METHODS

Tissue Culture Chambers, Nutrients, and Specimens: The sandwich-like multipurpose culture chambers (7, 8) incorporating the cellophane-strip technique (9) were used throughout these experiments. From top to bottom these chambers[1] consist of: (*a*) a metal retaining plate (2 × 3 or 4 × 4 inches) with a large central hole, (*b*) a No. 1 coverslip (45 × 50 mm. or 3 × 3 inches), (*c*) a latex rubber or silastic (silicone) gasket the size of the coverslip (1/8 to 3/8 inches thick) with a large hole corresponding in size to that of the metal plates, (*d*) a second coverslip, and (*e*) a second retaining plate. The five layers are firmly held together by four Allen-headed bolts and this forms a chamber of two parallel coverslips separated by a circular gasket wall. Sheets of Visking dialysis cellophane were prepared according to previously indicated techniques (9); however, in this case only the large sheets which completely covered one coverslip were used. These sheets, therefore, isolated the explants from the main nutrient vault. The explants were interposed between the first coverslip and the sheet of cellophane and this combination (coverslip-explant-cellophane) was interposed between a retaining plate and the gasket. In this way all explant nutrition was a dialysate of the standard laboratory nutrient (9). The explants (1 × 2 mm.) were cut from the mid-third of the shaft of 13 to 16 day embryo chick leg bones (3) after scraping out the marrow and peeling off the periosteum.

[1] Supplied by McCreary Manufacturing Company, Houston, Texas.

Phase Contrast Equipment: Records were made on ektachrome commercial film with an EMDECO[2] time-lapse unit fitted to a Kodak cine special 16 mm. camera (10) and on Agfa IFF 13 panchromatic film with a Hasselblad 1000F (2¼ × 2¼ inch) still camera, both being coupled to Bausch and Lomb phase contrast microscopes incorporating long working distance condensers.

Cytochemistry: The chambers were evacuated and after making several perforations in the cellophane with a No. 25 hypodermic needle, the cultures were washed with balanced salt solution (BSS) and fixed in the chambers with cold Earle's formol (5°C. for 15 minutes). The chambers were then opened by removing the screws and carefully separating coverslips from gaskets, the cellophane pulled away, and the culture containing coverslips washed in distilled water. Explants prepared in this way were treated for alkaline and acid phosphatase reactions by Gomori's techniques (11, 12), substrate incubation time being 15 to 30 minutes in both cases; for polysaccharides and for mucopolysaccharides (after saliva digestion for 30 minutes at 37°C.) according to the periodic acid–Schiff (PAS) method of Hotchkiss (13); for RNA with Unna's methyl green–pyronine B staining reaction (including RNase controls) according to Brachet (14); and for neutral fat by a 20 minute immersion in carbitol (diethylene glycol monoethyl ether) saturated with sudan IV (15). All stained preparations were water washed and mounted directly with glycerin jelly.

Vital Staining: Since the main nutrient vault was separated from the very thin cultivation vault by the dialysis membrane, vital staining was often very slow. A drop or two of a 1 per cent aqueous solution of neutral red, methylene blue, or Janus green was injected through the gasket into the main nutrient vault with the needle and syringe technique, vital staining occurring variously in 30 minutes to 4 hours as the dye slowly penetrated the cellophane membranes (Visking dialysis tubing has an average pore size of 24 A). Additional injections were sometimes necessary. As staining commenced to be faintly obvious the nutrient containing the dye was removed, the chamber rinsed with BSS, and then refilled with fresh nutrient or fresh BSS. In this way the dyes were brought to the living cells very gently.

RESULTS

Morphology and Physiology

Morphology: Primary emigrations (Figs. 1 and 3) from the bone fragments were spindle and kite-shaped fibroblastoid cells with oval nuclei. In 4 to 5 days these cells had moved peripherally and often contained numerous mitoses. Close to the nuclei in some of these cells were oval or round areas of white droplets and these were generally on one side of the nucleus. As cells advanced in age, highly refractile fat droplets often crowded the cytoplasm but did not accumulate in these juxtanuclear areas.

In 4 to 6 days a secondary emigration (osteoblasts) was observed and consisted of phase darker cells which generally remained close to the bone fragments (Figs. 1 through 4). These cells did not become plentiful until after 2 or 3 weeks of cultivation. Nuclei of the osteoblasts were rounder and the cytoplasm contained phase gray–black granules of varying size (0.5 to 20 micra). Two categories have been recognized: (*a*) large granules (*lg*) which are phase gray or phase black (generally 1 to 20 micra), and (*b*) small granules (*sg*) which are phase black (generally 0.5 to 2 micra). Varying numbers of the small granules were frequently lodged against the surfaces of the large granules (Figs. 5, 24, 36, 37, 43, and 45).

A very prominent phase gray juxtanuclear body which usually contained white droplets was observed in the majority of these osteoblasts (Figs. 2, 4, through 8). These bodies were approximately the size of the nucleus, being flat or discoidal in thinly spread cells (Figs. 2 and 6) and spheroidal in thicker cells (Figs. 4, 7, and 8). It will now be referred to as the Golgi complex (*Go*). Rarely an osteoblast was observed with two or three nuclei (Figs. 7 and 8), but none of these contained more than one Golgi complex. In some cells the apparently rough surfaces and/or spaghetti-like forms of the complex were more easily delineated. During secretory cycles the phase white droplets (approximately 0.25 to 5.0 micra) located at various levels within and upon the surfaces of these juxtanuclear bodies were microscopically observed to move, slowly. Often upon the surfaces of these phase white droplets tiny (approximately 0.3 micra) phase dark granules (*tg*) could be observed moving rapidly in distorted orbital paths (Figs. 7, 8, and 36). Most of the photographs do not show this granule-droplet complex as time exposures did not sufficiently freeze their motion.

Physiology-Droplet Emanation: Using time-lapse photographic methods with the still camera, droplet activity in the Golgi complex of the cell in Fig. 6 was followed for 62 minutes. Minute

[2] Supplied by the Electro-Mechanical Development Company, Houston, Texas.

droplets often coalesced or enlarged in an undetectable way and finally emerged into the cytoplasm (Figs. 9 through 23). Although this activity could be observed with the microscope, it was usually so slow that the accelerating effect of time-lapse cinematography was necessary to assure the direction of the droplet movement and to denote the abundance of the emerging forms. Specific droplets (*A*, *B*, *C*, etc.) derived from this Golgi complex are indicated in the legend for Figs. 9 through 23. In no instances were droplets released from the Golgi complex of this cell (or of the many cells followed in motion picture films) observed to pass through cell membranes or to be eliminated from the cytoplasm by any means. Once in the cytoplasm they aggregated with other phase light and phase dark granules. The most consistent impression from the many motion picture films studied was that these Golgi released droplets became surrounded by numerous phase black small granules (*sg*) already present in the cytoplasm as well as the tiny granules (*tg*) they possessed as they emerged from the Golgi body and then became lost from view. This transition, from their appearance in the Golgi complex until they disappeared in the cytoplasm, generally lasted 1 to 4 hours.

Cytochemistry and Vital Dyeing

As pointed out earlier, the osteoblasts were slow to appear so that 2 or 3 weeks elapsed before satisfactory groupings could be studied. Therefore, the following data were obtained from cells of emigration in cultures incubated for approximately 3 weeks. These are summarized in Table I.

Alkaline Phosphatase: The phase white droplets of the Golgi complex frequently were densely stained; whereas, the Golgi body reacted negatively or was weakly positive. The phase black small granules (*sg*) so often attached in varying numbers to the large granules (*lg*) also generally reacted with a strong positive, while the large granules reacted less strongly in some cells, negatively in others. The nucleoli were always more positive than the nuclei, both being variably positive from cell to cell (Figs. 24 and 25). The nucleus was sometimes negative.

Acid Phosphatase: As the tabulation in Table I shows, the spectrum of this analysis was similar to, though somewhat less marked than, that for the alkaline phosphatase. The Golgi complex (droplets and body) was negative. The cytoplasmic large granules reacted equivocally and the small granules negatively. The nucleolus was usually a strong positive, and the nucleus reacted somewhat less than the nucleolus, sometimes negatively.

RNA: This technique afforded a consistent and universal reaction. The Golgi droplets and phase black small granules in the cytoplasm were stained a distinctive deep red by the pyronine (Figs. 26 through 31) but after ribonuclease digestion failed to react. All other elements of the cells were negative. Even the nucleoli had only an occasional portion of its form stained so that it was impossible to tell whether the reaction was due to the nucleolus or a superimposed small granule.

Sudan IV: This orange reaction could not be resolved in any of the six components listed in Table I and was present only in the rarely observed cytoplasmic fat droplets. Of course, many fibroblastoid cells located peripheral to the osteoblasts contained an abundance of positively reacting fat droplets.

Mucopolysaccharides - Polysaccharides: The large granules in the cytoplasm gave the only significant response to both PAS reactions. The Golgi complex gave a negative reaction for the mucopolysaccharides but the secretory droplets showed an occasional coloration for polysaccharides in general but not for mucopolysaccharides. The nucleus and nucleolus were negative, and the small granules could not be discerned in bright field as the strong color reaction of the large granules impaired resolution (Figs. 32 through 35).

Neutral Red: The Golgi complex (droplets and body) was unstained. However, upon the surfaces of the emanating droplets one or several of the tiny granules (*tg*) could be observed as a red density in a bright field. In the cytoplasm the large granules were lightly stained and the small granules adjacent to, or upon, the large granules were deeply colored (Figs. 36 through 39).

Methylene Blue: The Golgi droplets, cytoplasmic granules (*lg* and *sg*) and the nucleoli stained with this vital dye. The display of the Golgi droplets with phase contrast was green even after they had emerged into the cytoplasm; whereas, the large granules (*lg*) appeared red. The brightfield observations revealed all positive reactions as a blue coloration (Figs. 40 through 48).

Janus Green: Vital staining only revealed a coloration in the mitochondria. Dense and pro-

Explanation of Figures

Ex, bone explant
Os, osteoblasts
Fb, fibroblasts
Go, Golgi complex
N, nucleus
Ns, nucleolus
sd, secretory droplets in and emanating from the Golgi complex
lg, large granules in the cytoplasm
sg, small granules frequently attached to the large granules in the cytoplasm
tg, tiny granules found in or on the secretory droplets of the Golgi complex
A, *B*, *C*, *D*, *E*, *F*, *G*, *H*, *J*, *R*, and *b*, specific secretory droplets in Figs. 9 to 23
Mit, mitochondrion
The encircled *A*, *B*, *C*, and *D*, specific osteoblasts in Figs. 24 and 25
Alk ph, positive alkaline phosphatase staining reaction
RNA, cytoplasmic and Golgi complex granules reacting positively to RNA staining
m, minutes, interval of time between sequence photographs

FIGURES 1 through 8

Phase contrast photographs of osteoblasts from embryo chick leg bone fragments cultivated under full sheets of cellophane in multipurpose culture chambers. Magnification line for Figs. 1 and 3 is in Fig. 1; for Figs. 2, 4, 5, 7, and 8 in Fig. 2; and for Fig. 6 in Fig. 6.

FIGURES 1 and 3

Low power views of bone explants (*Ex*), the juxtapositioned osteoblasts (*Os*), and the more peripheral fibroblastoid (*Fb*) elements. The arrows which join Fig. 1 with Fig. 2 and Fig. 3 with Fig. 4 associate specific osteoblasts. In Fig. 1 the more peripherally located fibroblastoid cells contained large refractile fat droplets. × 135.

FIGURES 2 and 4

High power photographs of two osteoblasts from Figs. 1 and 3 showing in greater detail the Golgi complex (*Go*) and secretory droplets (*sd*). × 2200.

FIGURE 5

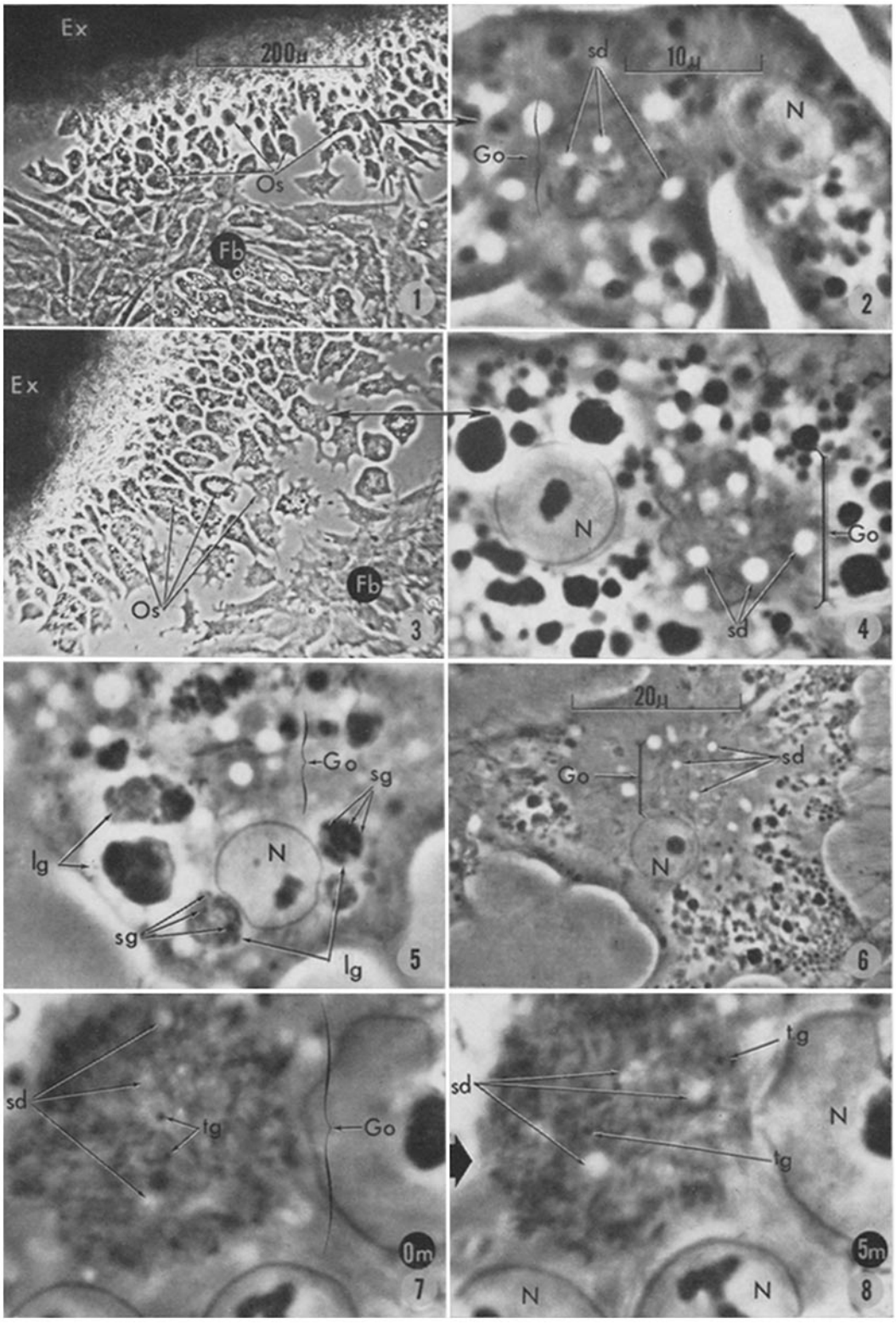
High power view of cell *A* from Fig. 24 showing the relationship of small granules (*sg*) to large granules (*lg*). × 2200.

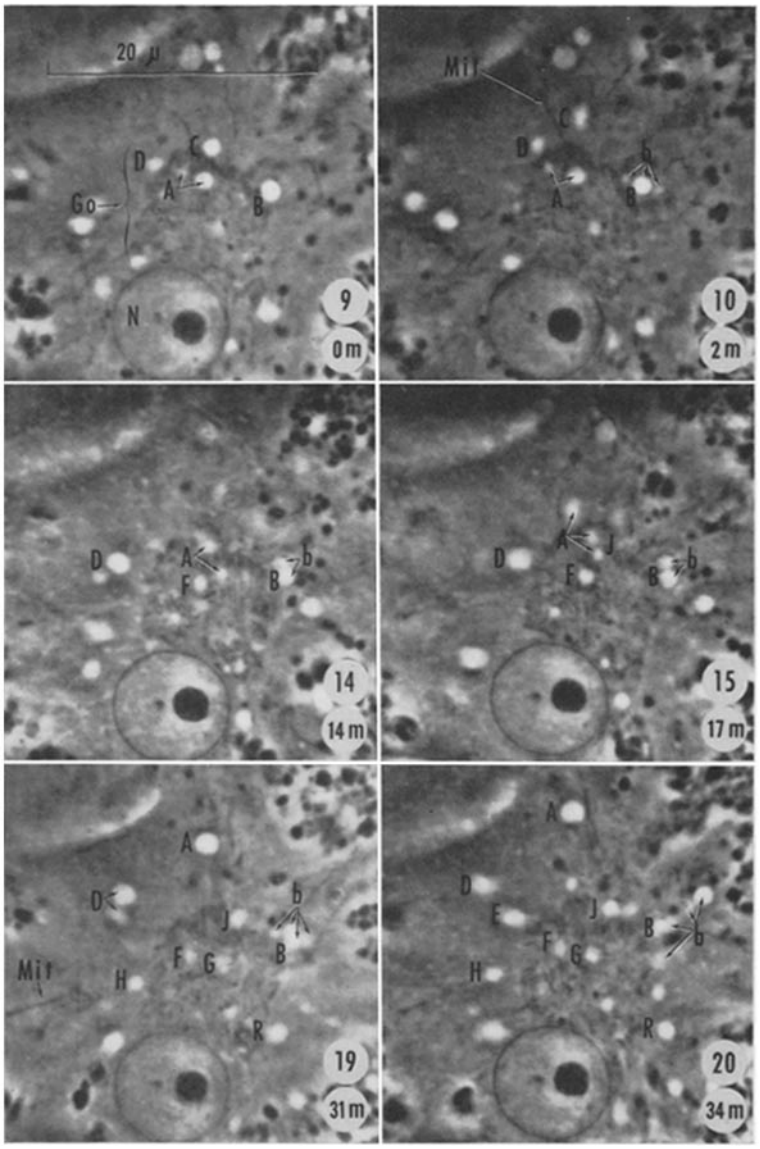
FIGURE 6

A very thinly spread osteoblast resulting from the closely apposed dialysis cellophane membrane which quite isolated the Golgi complex from the cytoplasmic granules. Fine filaments of this Golgi complex were somewhat smaller than the mitochondria. The secretory droplet (*sd*) activity of this cell is shown in Figs. 9 through 23 at a higher magnification. × 1350.

FIGURES 7 and 8

High power photographs of a portion of an unusually large trinucleated osteoblast which contained a single Golgi complex. Compare the nuclear and Golgi complex size of this cell with those in Figs. 2, 4, and 5 reproduced at the same magnification. The stringy character of this Golgi complex, the emanating secretory droplets (*sd*), and the tiny granules (*tg*), frequently associated with them are shown. There was a change in the topography of this Golgi structure during the 5-minute interval. (0*m* to 5*m*). × 2200.


Ex
200μ
Os
Fb
1
sd
10μ
N
Go
2
Ex
Os
Fb
3
N
Go
sd
4
Go
sg
lg
N
sg
lg
5
20μ
Go
sd
N
6
sd
tg
Go
0m
7
sd
tg
N
tg
5m
N
N
8



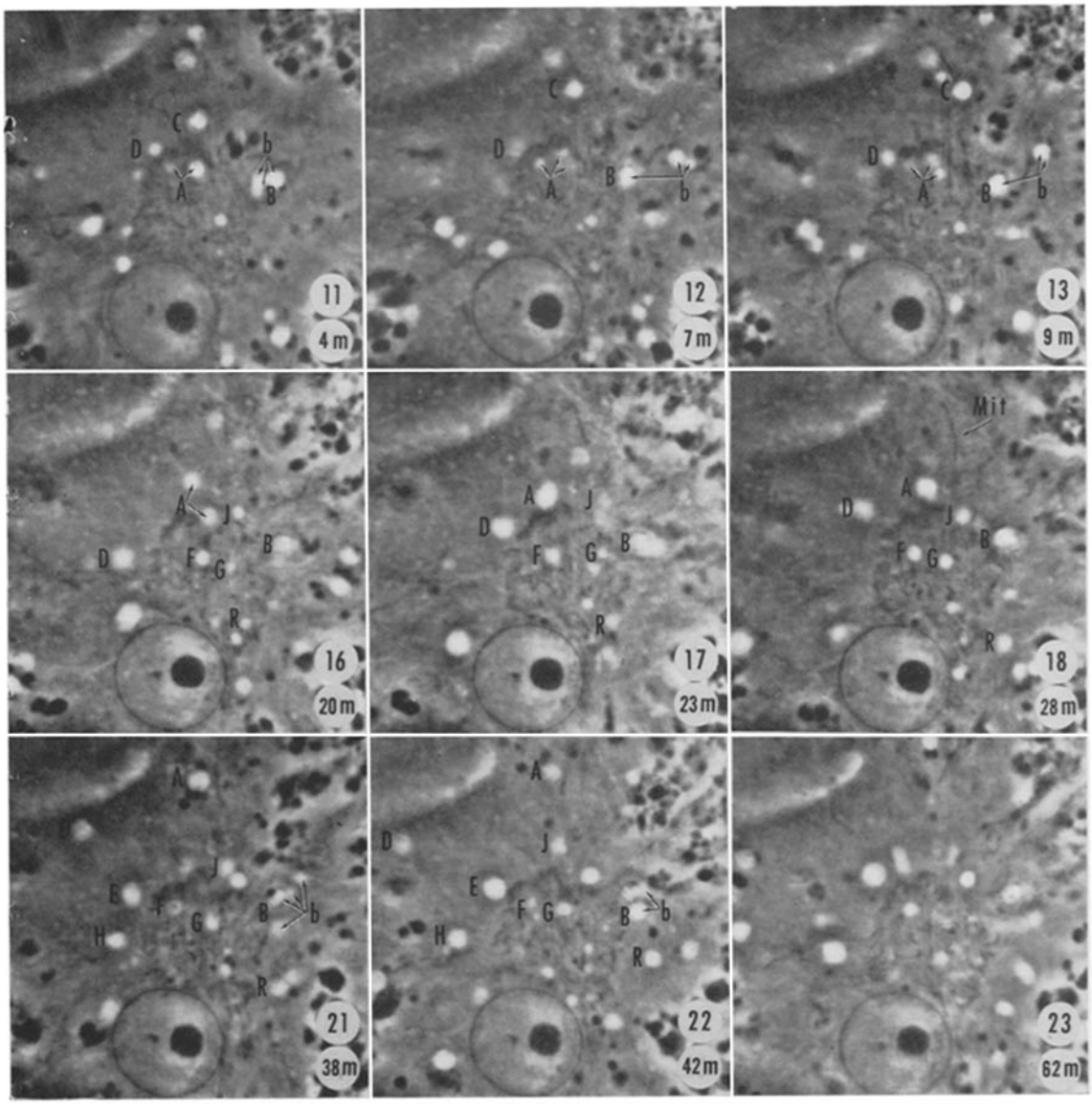
FIGURES 9 through 23

Phase contrast photographs of the central portion of the cell of Fig. 6 showing the nucleus, (*N*), mitochondria, (*Mit*), Golgi complex, (*Go*), and emanating secretory droplets (*A*, *B*, *C*, *D*, *E*, *F*, *G*, *H*, *J*, *R*, and *b*). Magnification line for these photographs is in Fig. 9. Secretory droplet *A* is shown as two droplets in Figs. 9 through 11, 14 and 16; as three droplets in Fig. 12, 13, and 15; and as a single coalesced droplet which emerged from the Golgi complex in Figs. 17 through 22. Droplets *B* and *b* were located in an active area of this Golgi complex as numerous emergences took origin from this point. Similarily the other droplets may be followed over this 62-minute interval (0*m* to 62*m*). × 1850.

longed staining colored the small granules (*sg*) slightly.

DISCUSSION

The data of this report were presented (*a*) to illustrate an unequivocal demonstration of the long debated secretory cycle of the Golgi complex, and (*b*) to define the cytochemistry of this complex and associated structures in one cell type, the chick osteoblast. These observations supported the concepts of workers who over the past 60 years had postulated such an activity.



Having observed phase white droplets in this juxtanuclear body, it was a simple task to determine their exodic direction of movement, and their coalescing and segregating activities by time-lapse photography.

Structurally, the Golgi complex observed with phase contrast appeared gray and the emerging droplets white. When the complex was not surrounded by cytoplasmic granules and droplets, it often appeared in the form of a loose "ball of yarn." Janus green did not stain the strands of such forms indicating they were probably manifestations of the parallel membranes observed with electron microscopy rather than intertwining mitochondria. A reconstruction of the Golgi complex from EM photographs will depict its form as a loose discoidal or spheroidal network with large and small droplets especially comparable to that in Figs. 7 and 8. These net-like forms are likely equivalent to the frequently debated canalicular structures of classical Golgi techniques. Moreover, the Golgi structure apparently is not a rigid and unchangeable form. Figs. 7 and 8 and the sequence in Figs. 9 through 23 illustrate topographic changes occurring during secretory cycles. These changes, of course,

were more pronounced in time-lapse cine records in which the speed of life of the cells was accelerated 960 times (1 frame per minute). Golgi forms would not only undergo contour changes but drastic size alterations as well.

To further relate this juxtanuclear body to the Golgi complex defined by others, a number of cytochemical and vital dye reactions were made. Earlier workers (16, 17) indicated that the Golgi complex in some cases reacted positively for alkaline phosphatase. More recent data (Kuff and Dalton, 18) determined by cellular fractionation indicated that only lipid phosphorus and acid phosphatase activities were concentrated in the Golgi fraction and this agreed with other recent histochemical studies which had shown a high acid phosphatase activity (19, 20) and a low or absent alkaline phosphatase activity in the Golgi region of epididymal cells (19, 21). In this analysis of Kuff and Dalton a high alkaline phosphatase activity was found in the layer just under the Golgi fraction, but this contained few Golgi particles when viewed with the electron microscope. The absence of a positive acid phosphatase but high incidence of a positive alkaline phosphatase in the Golgi droplets of the osteoblasts probably represents a cytological difference between the cells studied rather than a discrepancy in these various reports. It seems reasonable to consider the enzyme machinery as a likely area of important cellular distinctions.

Earlier studies of Schneider *et al.* (22) had shown a high RNA and alkaline phosphatase activity in the Golgi complex of epididymal homogenates. However, Kuff and Dalton's more recent report showed only a small RNA fraction in the Golgi layer. The positively reacting Golgi droplets, therefore, may have been an artifact due to a superimpositioning of the small RNA granules upon the surfaces of the Golgi complex. An emission of RNA with the droplets from the Golgi body, therefore, cannot be substantiated. Since Palade (23, 24) has shown a continuity of the endoplasmic reticulum (ER) with the Golgi membranes, the nuclear membranes (25), and cell membranes, the Golgi complex would have access to the nucleolus through the ER's continuity with the nuclear membranes. This ER-Golgi-nucleus relationship indicates that RNA synthesis could be an integrated activity of all three. Autoradiographic studies are being initiated to further investigate this interrelationship.

The negative PAS reaction of the Golgi complex (body and droplets) was contrary to the work reported by Leblond (26) and Bensley (27) but this again, along with the absence of a lipid response, probably was due to a cytological distinction of the material studied.

The methylene blue vital dyeing was corroborative of Dalton's recent work (6) as well as that of a whole array of previous reports. The phase coloring of the methylene blue (green for Golgi droplets, red for large granules in the cytoplasm) gave a very useful separation for color cinematography. In the comparative study of freshly obtained and electron microscopically observed cells, Dalton and Felix (5) indicated the Golgi complex to be refractory to vital dyeing with

FIGURES 24 and 25

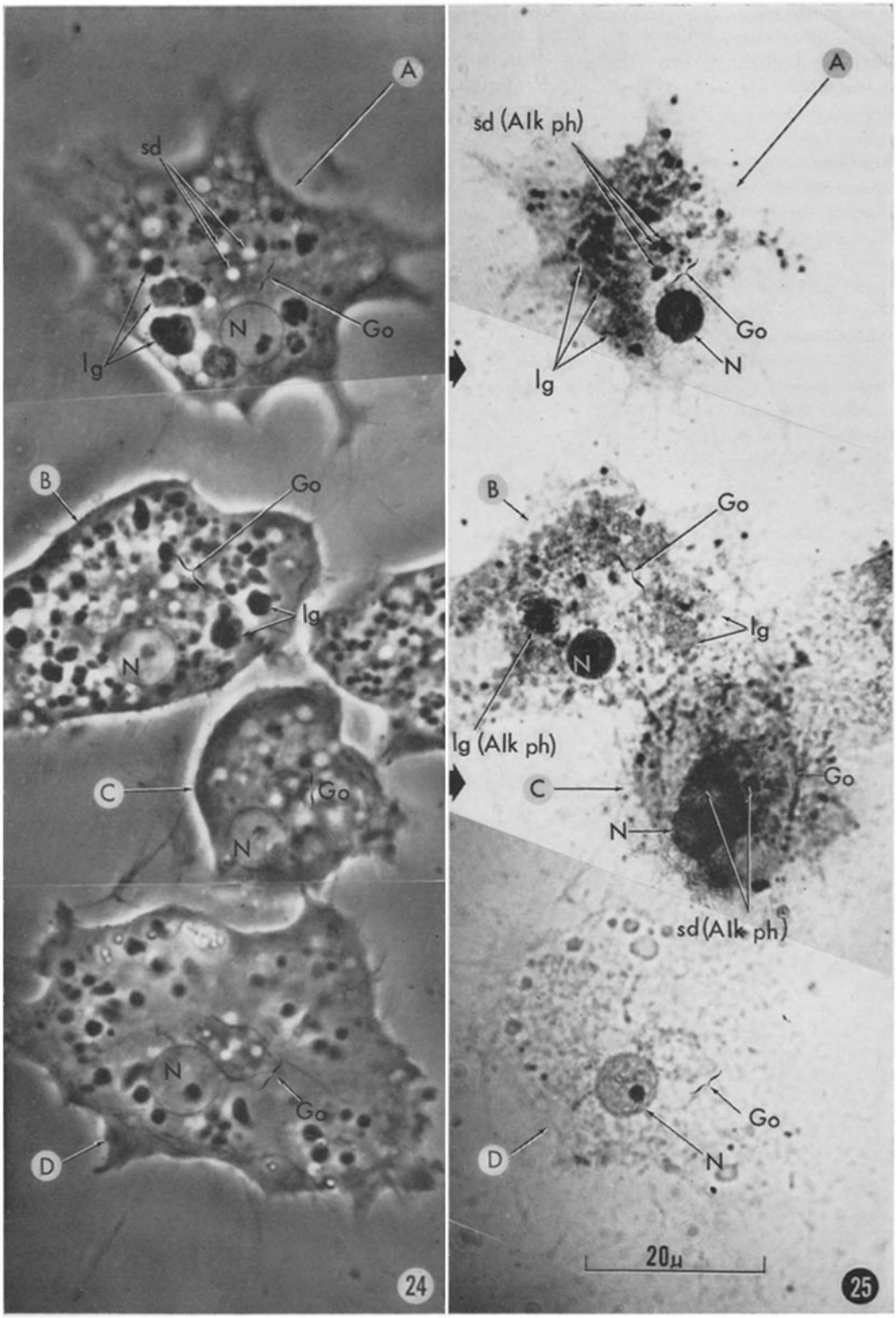
Phase contrast and parallel bright field photographs of living and alkaline phosphatase stained osteoblasts (the encircled *A*, *B*, *C*, and *D*) which had emigrated from an embryo chick bone fragment cultivated under cellophane in the multipurpose culture chamber. Magnification line for Figs. 24 and 25 is in Fig. 25 × 1400.

FIGURE 24

The Golgi complexes (*Go*) of all four osteoblasts were in an active secretory phase as indicated by the secretory droplets (*sd*).

FIGURE 25

Bright field photograph of the cells shown in Fig. 24 after they had been fixed in formol and stained for alkaline phosphatase. The secretory droplets (*sd*) (*Alk ph*) of cells *A*, *B*, and *C* showed a marked alkaline phosphatase reaction; whereas, those of cell *D* were negative. The nucleoli and nuclei (*N*) of all four of these cells were positive for the alkaline phosphatase reaction. The large granules (*lg* or *lg* (*alk ph*)) and small granules upon the large granules particularly of cells *A*, *B*, and *C*, were variably positive.


A
sd
N
Go
lg
B
Go
lg
N
C
Go
N
D
N
Go
24
A
sd (Alk ph)
Go
N
lg
B
Go
lg
N
lg (Alk ph)
C
Go
N
sd (Alk ph)
Go
D
N
20μ
25

neutral red. The Golgi complex of the osteoblasts in this report was comparable with their observation,

In discussing the morphology of secretion in Frontiers in Cytology Palay (28) referred to phase contrast photomicrographs of sarcoma cells with finely granular juxtanuclear masses suggested by the authors (Ludford *et al.*, 29, 30) to be the Golgi apparatus. Palay concluded by saying, "the great majority of authors have not reported seeing anything resembling the Golgi apparatus in living or surviving cells." This raises the following question: Why do osteoblasts as depicted in this presentation demonstrate such vivid juxtanuclear bodies consistent with the Golgi complex? Many workers have observed juxtanuclear zones into which lipoidal or protein granules of the cytoplasm did not aggregate and have presumed these "empty" areas to be negative outlines of the Golgi complex. Others have determined such negatively outlined areas to be the Golgi complex since cytochemical techniques stained this juxtanuclear round form. It is believed that the method utilized in the preparation of these cultures significantly contributed to a visualization of cells in a highly differentiated form. A number of studies carried out in this and other laboratories (9, 31–35) have demonstrated the usefulness of the cellophane technique for the maintenance of emigrating cells in their functioning forms.

As a partial substantiation for this particular endeavor, there was a study by Barrett *et al.* (36) published in 1944 on a transplantable osteogenic sarcoma which originated in a mouse. In the early generations the Golgi body of this tumor was very pronounced, the growth slow, osteogenesis active, and the alkaline phosphatase reaction quite high. As the tumor was transplanted through several passages the alkaline phosphatase became quite low or absent, the growth rate increased but the osteogenic activity diminished along with the size of the Golgi complex. Barrett concluded that the presence of a very pronounced Golgi complex, therefore, was indicative of osteogenic activity, and its reduction a loss of functioning. Much earlier (1936) a similar decision had been reached by Hill (37) from sections of *in vitro* derived osteoblasts. She concluded, "There is a close relationship between the forms of the Golgi apparatus and that state of differentiation and functional activity of the osteoblasts; it is probable that there is a relation between the production of the intercellular substance of the bone, and the condition of the Golgi material." Other supportive evidence for appreciating the presence of a well formed Golgi complex as indicative of normal cellular activity is offered in the work on functioning adenomas by Haguenau and Bernhard (38) and for a diminished or vestigial complex as indicative of a loss of functioning in the work of Dalton and Felix (39) and Howatson and Ham (40) on anaplastic tumor cells.

Since the cellophane strip technique has been shown to be conducive to the functioning of other

FIGURES 26 through 31

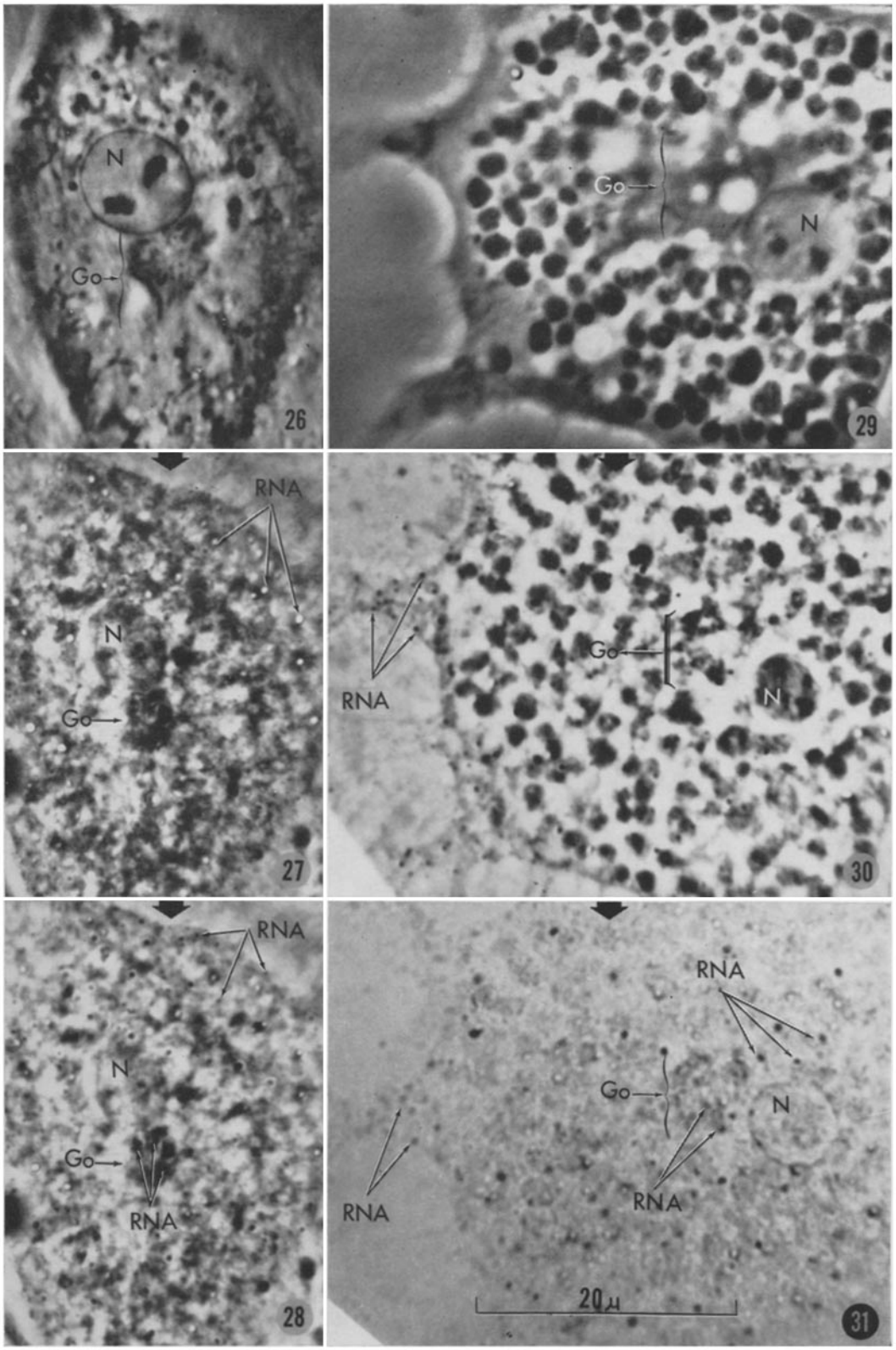
Phase contrast and parallel bright field photographs of two embryo chick osteoblasts cultivated under cellophane in a multipurpose culture chamber. Magnification line for Figs. 26 through 31 is in Fig. 31 × 2100.

FIGURES 26 through 28

Fig. 26 shows the osteoblasts by phase contrast microscopy in the living state, Fig. 27 after fixation with formol and subsequent RNA staining, and Fig. 28 by bright field microscopy after RNA staining. The RNA positive granules (*RNA*) of the cytoplasm were observed as green granules by phase contrast microscopy but appear white in the peripheral part of the photographs and dark in the Golgi complex (*Go*). With bright field the RNA granules (*RNA*) appeared a luminous red.

FIGURES 29 through 31

Figs. 29 and 30 are phase contrast photographs of one osteoblast, first in the living state and then after formol fixation and RNA staining. Fig. 31 is a bright field photograph of the same cell. The RNA granules (*RNA*) in the cytoplasm and in the Golgi complex (*Go*) are indicated.


N
Go
26
Go
N
29
RNA
N
Go
27
RNA
Go
N
30
RNA
N
Go
RNA
28
RNA
Go
N
RNA
RNA
20μ
31

cell types, it follows that the osteoblasts cultivated in an equivalent environment should contain a morphologically and physiologically prominent Golgi complex.

Although the data of this report define a droplet emanation from juxtanuclear phase gray bodies and enumerate certain cytochemical reactions of the nucleus and cytoplasmic organoids in one cell type in tissue culture, they must not be construed as evidence for a universal pattern of cellular activity. There appears to be a wide range of variation in the cytochemical reports on the Golgi complex, yet, in most of these reports, different cell types were investigated. Important clues to cytochemical differentiation have, thereby, been established. Further, this study cannot be considered conclusive as many interesting doors were only opened for investigation. For instance, the interrelationship of the phase white Golgi droplets (*sd*) and tiny phase black granules (*tg*) which were often upon them before they had left the Golgi body suggests a similarity to the reverse phenomenon of pinocytosis in which incoming droplets were joined to microkinetospheres (41). These, too, encircled the fluid droplets in orbital paths and aggregated in amounts commensurate with the size of the pinocytosed droplets. Second, a true relation was not demonstrated between the

FIGURES 32 through 35

Phase contrast and parallel bright field photographs of three osteoblasts cultivated from embryo chick bone fragments under cellophane in the multipurpose culture chamber. These cells were formol fixed and stained for mucopolysaccharides. Magnification line for Figs. 32 through 35 is in Fig. 37. × 1350.

FIGURES 32 and 33

Fig. 32 is a phase contrast photograph and Fig. 33 is a bright field photograph of an osteoblast showing the PAS reaction in the large granules (*lg*). The nucleus (*N*) and Golgi complex (*Go*) were negative to the PAS stain.

FIGURES 34 and 35

Fig. 34 is a phase contrast photograph and Fig. 35 a bright field photograph of two adjacent osteoblasts. The two secretory droplets (*sd*) from the Golgi complex (*Go*) of the cell on the left are shown to be negatively reacting to the PAS stain whereas the large granules (*lg*) in all the cells reacted positively.

FIGURES 36 through 39

Phase contrast and parallel bright field photographs of living osteoblasts vitally stained with neutral red. The cells were cultivated from embryo chick bone fragments under cellophane in the multipurpose culture chamber. Magnification line for Figs. 36 through 39 is in Fig. 37. × 1350.

FIGURES 36 and 37

The osteoblast in Fig. 36 was photographed through the phase contrast microscope and in Fig. 37 through the bright field microscope to show the location of the neutral red reaction in the living cell. The tiny granules (*tg*) frequently associated with the secretory droplets emanating from the Golgi complex (*Go*) are shown to stain vitally with neutral red as well as the small granules (*sg*) and large granules (*lg*) of the cytoplasm. The Golgi complex, secretory droplets, nucleus, and nucleoli were refractory to neutral red.

FIGURES 38 and 39

This osteoblast is shown after vital staining with neutral red in Fig. 38 under phase contrast and in Fig. 39 under bright field microscopy. The Golgi complex (*Go*) and secretory droplets were refractory to the neutral red.

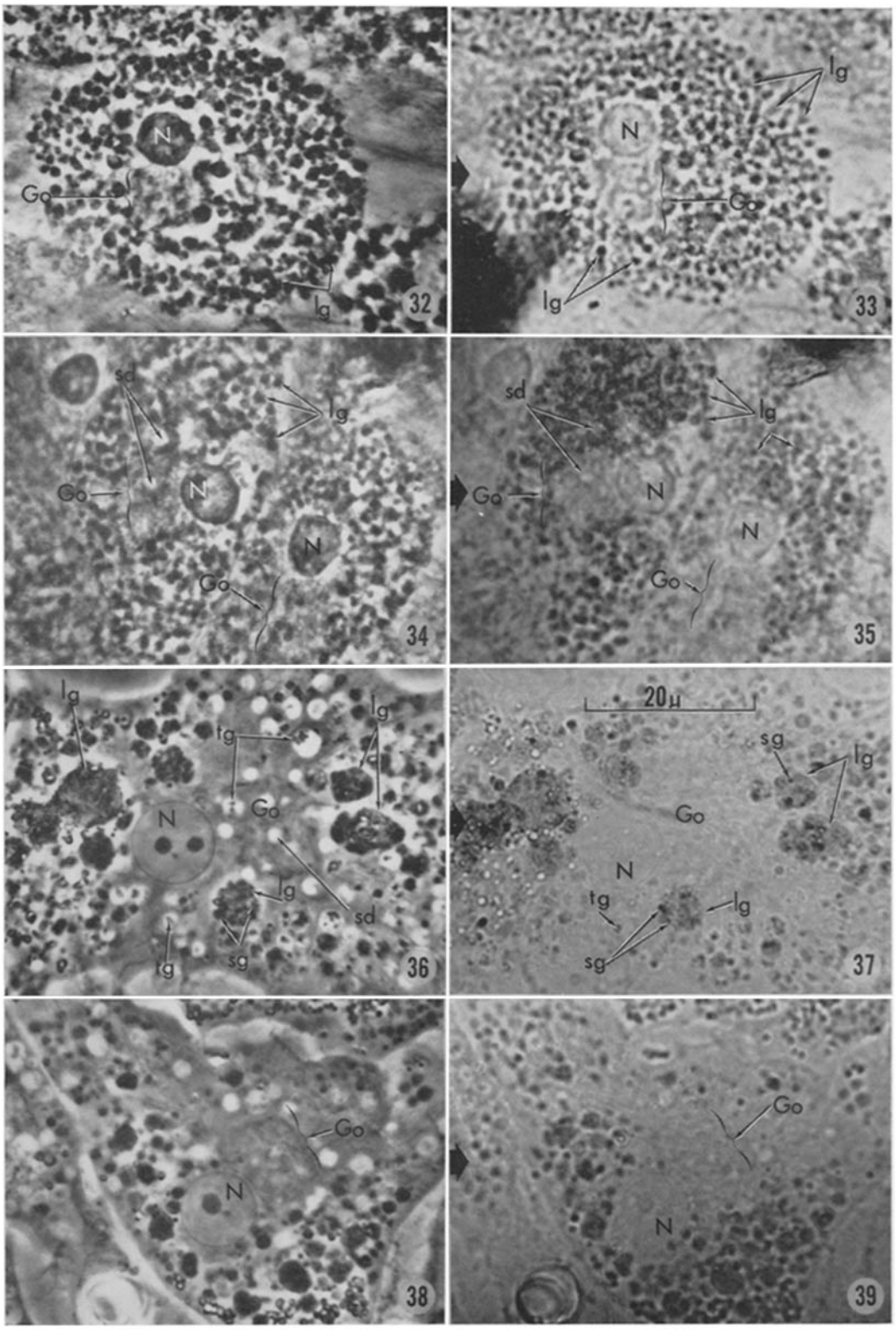

N
Go
lg
32
lg
N
Go
lg
33
sd
lg
Go
N
N
Go
34
sd
lg
Go
N
N
Go
35
lg
tg
lg
N
Go
lg
sd
tg
sg
36
20μ
sg
lg
Go
N
tg
lg
sg
37
Go
N
38
Go
N
39

TABLE I

Cytochemical Responses of Embryo Chick Osteoblasts

Analysis	Golgi Droplets (phase white)	Golgi body (phase gray)	Large granules (phase gray-black)	Small granules (phase black)	Nucleolus (phase black)	Nucleus (phase gray)
			(*1–20μ*)	(*0.5–2μ*)		
Alkaline phosphatase	±+	±	±	±+	++	±
Acid phosphatase	−	−	±	−	++	±
RNA (Unna-Brachet)	++	−	−	++	−	−
RNA (RNA-ase digestion)	−	−	−	−	−	−
Sudan IV (Gomori)	−	−	−	−	−	−
PAS (polysaccharide)	+	−	+	Not clearly resolved	−	−
PAS (mucopolysaccharide)	−	−	+	Not clearly resolved	−	−
Neutral red	−	−	+	++	−	−
Methylene blue	+	−	+	++	+	−
Janus green	−	−	−	±	−	−

\+ = Reaction ± or ±+ = Reaction equivocal
++ = Strongest reaction − = No reaction

Golgi released droplets and other forms in the cytoplasm, and whether the RNA was a part of the phase white droplet or the tiny granule upon its surface may only be understood and appreciated as more vigorous studies are made and related to function.

This work was supported in part by a grant-in-aid from the American Cancer Society, Inc.; a grant-in-aid from The Orthopaedic Research and Education Foundation; a grant-in-aid from the Easter Seal Research Foundation; by the United States Army Medical Research and Development Command, Department of the Army, under Contract No. DA-49-193-MD-2087, administered by C. M. Pomerat; and by Research Grant (C-5100) from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

Received for publication, August 20, 1960.

FIGURES 40 through 48

Phase contrast and parallel bright field photographs of three living osteoblasts from embryo chick bone fragments cultivated under cellophane in the multipurpose culture chamber. Magnification line for Figs. 40 through 48 is in Fig. 40. × 1350.

FIGURES 40, 43, and 46

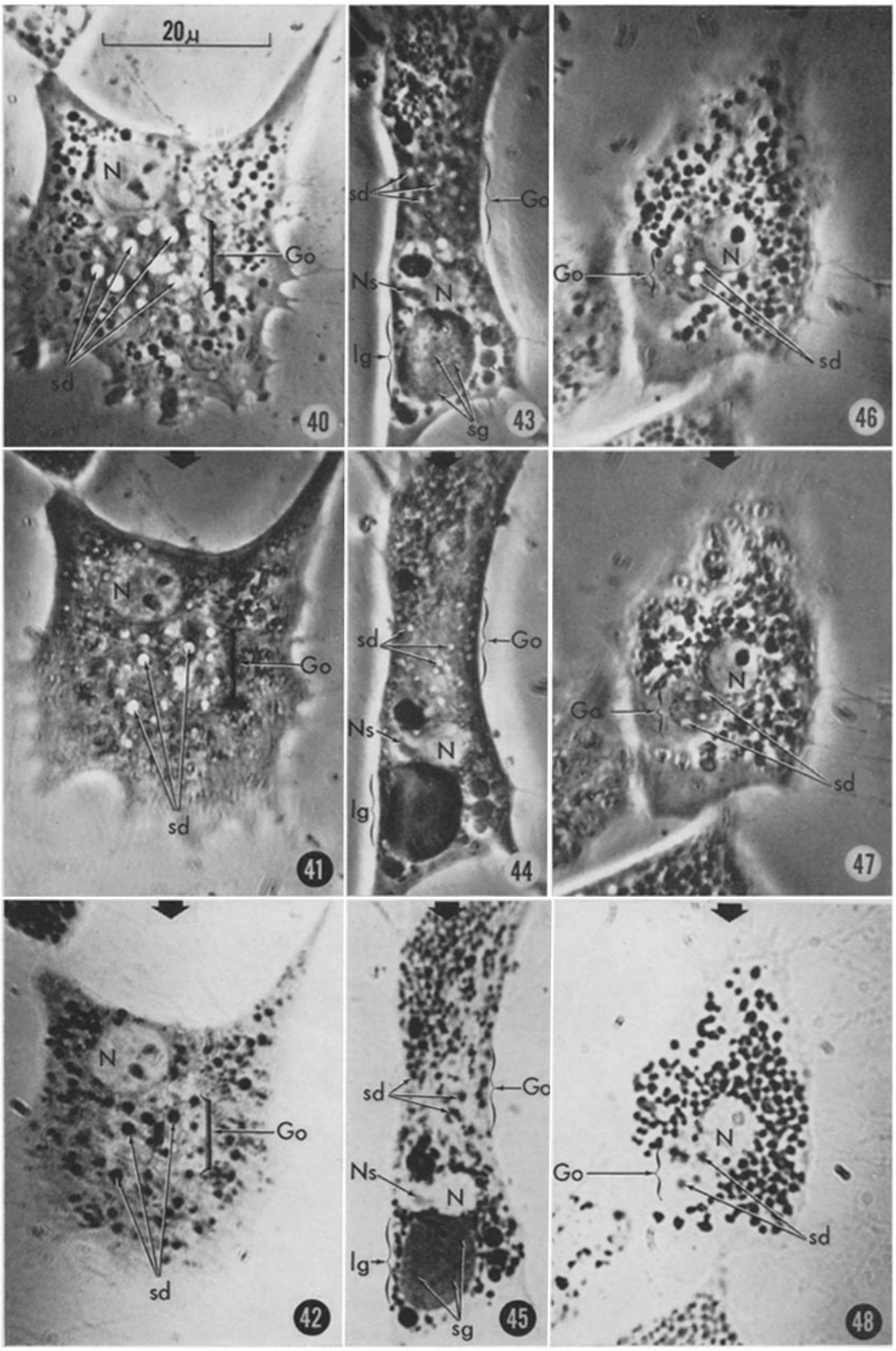
Phase contrast photographs of three osteoblasts before vital staining with methylene blue. The Golgi complex (*Go*), nucleus (*N*), secretory droplets (*sd*), large granules (*lg*), small granules (*sg*), and nucleolus (*Ns*) are shown.

FIGURES 41, 44, and 47.

Phase contrast photographs after vital staining with methylene blue. The secretory droplets appeared a brilliant green and the large granules a dull red.

FIGURES 42, 45, and 48

Bright field photographs after vital staining with methylene blue. The nucleolus (*Ns*), secretory droplets (*sd*), large granules (*lg*), and small granules (*sg*), stained a rich blue.


20μ
N
Go
sd
40
sd
Go
Ns
N
lg
sg
43
Go
N
sd
46
N
Go
sd
41
sd
Go
Ns
N
lg
44
Go
N
sd
47
N
Go
sd
42
sd
Go
Ns
N
lg
sg
45
Go
N
sd
48

BIBLIOGRAPHY

1. SJÖSTRAND, F. S., and HANZON, P., *Exp. Cell Research*, 1954, **7**, 393, 415.
2. BRACHET, J., in Biochemical Cytology, (J. Brachet, editor), New York, Acadmic Press, Inc., 1957, 62.
3. ROSE, G. G., and SHINDLER, T. O., *J. Bone and Joint Surg.*, 1960, **42A**, 485.
4. ROSE, G. G., *J. Appl. Physics*, 1960, **31**, 1843.
5. DALTON, A. J., and FELIX, M. D., *in* Mitochondria and Other Cytoplasmic Inclusions, New York, Academic Press, Inc., 1957, 148.
6. DALTON, A. J., *in* Cell Physiology of Neoplasia, Austin, University of Texas Press, 1960, 161.
7. ROSE, G. G., *Texas Rep. Biol. and Med.*, 1954, **12**, 1074.
8. ROSE, G. G., *Texas Rep. Biol. and Med.*, 1957, **15**, 310.
9. ROSE, G. G., POMERAT, C. M., SHINDLER, T. O., and TRUNNELL, J. B., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 761.
10. ROSE, G. G., *Research Film*, 1960, **3**, 267.
11. GOMORI, G., *Stain Tech.*, 1950, **25**, 81.
12. GOMORI, G., Microscopic Histochemistry, 1952, 184.
13. HOTCHKISS, R. D., *Arch. Biochem.*, 1948, **16**, 131.
14. BRACHET, J., *Quart. J. Micr. Sc.*, 1953, **94**, 1.
15. GOMORI, G., *in* Methods in Medical Research, (M. B. Visscher, editor), Chicago, Year Book Publishers, 1951, 13.
16. DEANE, H. W., and DEMPSEY, E. W., *Anat. Rec.*, 1945, **93**, 401.
17. FREDERICSSON, B., *Exp. Cell Research*, 1956, **10**, 63.
18. KUFF, E. L., and DALTON, A. J., *in* Subcellular Particles, (Teru Hayashi, editor), New York, Ronald Press Co., 1959, 114.
19. MONTAGNA, W., *Ann. New York Acad. Sc.*, 1952, **55**, 629.
20. BURSTONE, M. S., *J. Nat. Cancer Inst.*, 1958, 21, 523.
21. MANEELY, R. B., *Acta Anat.*, 1955, **24**, 314.
22. SCHNEIDER, W. C., DALTON, A. J., KUFF, E. L., and FELIX, M., *Nature*, 1953, **172**, 161.
23. PALADE, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 567.
24. PALADE, G. E., *Anat. Rec.*, 1955, **121**, 445.
25. YASUZUMI, G., SUGIHARA, R., NAKANO, S., KISE, TOMO, and TAKEUCHI, H., *Cancer Research*, 1960, **20**, 339.
26. LEBLOND, C. P., *Am. J. Anat.*, 1950, **86**, 1.
27. BENSLEY, R. R., *Exp. Cell Research*, 1951, **2**, 1.
28. PALAY, S. L., *in* Frontiers in Cytology, (S. L. Palay, editor), New Haven, Yale University Press, 1958, 305.
29. LUDFORD, R. J., SMILES, J., and WELCH, F. V., *J. Roy. Micr. Soc.*, 1948, **68**, 1.
30. LUDFORD, R. J., and SMILES, J., *J. Roy. Micr. Soc.*, 1950, **70**, 186.
31. ROSE, G. G., Abstract, Tissue Culture Association Tenth Annual Meeting, Atlantic City, New Jersey, April 8–9, 1959, *Excerpta Med., Sect. I*, 1959, **13**, 222.
32. ROSE, G. G., *Texas Rep. Biol. and Med.*, 1960, **18**, 103.
33. POMERAT, C. M., and NADAZAWA, T., Abstract, Tissue Culture Association Tenth Annual Meeting, Atlantic City, New Jersey, April 8–9, 1959, *Excerpta Med., Sect. I*, 1959, **13**, 223.
34. CAPERS, C., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 559.
35. ROSE, G. G., and POMERAT, C. M., *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 423.
36. BARRETT, M. K., DALTON, A. J., EDWARDS, J. E., and GREENSTEIN, J. P., *J. Nat. Cancer Inst.*, 1944, **4**, 389.
37. HILL, J. C., *Arch. exp. Zellforsch.*, 1936, **18**, 496.
38. HAGUENAU, F., and BERNHARD, W., *Arch. anat. micr. et morphol. exp.*, 1955, **44**, 27.
39. DALTON, A. J., and FELIX, M., *Ann. New York Acad. Sc.*, 1956, **63**, 1117.
40. HOWATSON, A. F., and HAM, A. W., *Cancer Research*, 1955, **15**, 62.
41. ROSE, G. G., *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 697.