for those lines, which could also be detected by antiserum against the liver homogenate. Among these lines are those of the γ-globulin, marked 1 and 1', two of the β-globulin marked 2 and 3 (line 3 is saddle-shaped, forming a continuation of the line of proteins with more rapid mobility), line 4 and 5 belongs to the α_2-globulin fraction and line 7 and 8 to the albumin fraction. Finally, line numbered 9, which is visible before the line of albumin, can be stained by dyes used for the detection of lipoproteins.

In another experiment the results of which are shown in diagram 5, 6, 7 (and 11, 12, 13) of the Figure, we gave the results of the detection of serum proteins (of proteins of extract R) obtained with a specific antiserum No. 2425 (antiserum No. 428) and with the same antiserum after 24 h incubation with a sufficient amount of proteins of extract R (of rat serum) at laboratory temperature. It can be seen from the diagrams 5, 6, and 7, that extract R contains proteins capable by immunochemical reaction of inactivating antibodies of the specific antiserum against serum proteins, which produce the precipitation lines marked by numbers 1, 1', 2, 4, 5, 6, and 7. It can be seen from the diagrams 11, 12, and 13, that the serum also contains proteins, capable by immunochemical reaction of inactivating antibodies of the specific antiserum against proteins of the liver extract R, which can produce the precipitation lines marked by letters b, d, e, f, h, i, and j.

In order to prove identity between the single serum protein components and those liver proteins, which under the conditions of electrophoresis in Agar gave the same electrophoretic mobility, we used OUCHTERLONY's double diffusion method combined with transferring agar discs with the isolated fractions. Using these methods we succeeded in demonstrating the identity between the serum protein, forming line marked by number 2 and the protein of extract R, forming line marked by b.

In experiments to separate the proteins of liver extracts by means of electrophoresis in agar gel, 8–10 protein fractions could be detected. This is in keeping with our previous findings[5,7]. However, protein fractions of liver extracts with electrophoretic mobility of serum γ-globulin, or lower mobility, could not be demonstrated by the immunochemical reaction, although these proteins were present in both extracts and were well separated by electrophoresis in Agar. It should be considered that some liver proteins have antigenic groups, determining their immunochemical reaction, identical with some serum proteins. They may differ, however, in their physicochemical characteristics, such as electrophoretic mobility, diffusion rate, stability of their protein structure etc.

Diagram	Antigen	Rabbit antiserum
1	S	anti S (No. 137, 138, 177, 2425)
2	S	anti H (No. 1683, 1688, 1695, 2075)
3	S	anti D (No. 2040, 2908)
4	S	anti R (No. 428, 429)
5	S	anti S No. 2425 + saline $\overline{aa}$, incubated for 24 h at 20°C
6	S	anti S No. 2425 + 7% solution of R $\overline{aa}$, incubated for 24 h at 20°C
7	Difference of diagram 5 and 6	
8	D	anti S (No. 137, 138, 177, 2425)
9	D	anti H (No. 1683, 1688, 1695, 2075)
10	R	anti S (No. 137, 138, 177, 2425)
11	R	anti R No. 428 + saline $\overline{aa}$, incubated for 24 h at 20°C
12	R	anti R No. 428 + serum $\overline{aa}$, incubated for 24 h at 20°C
13	Difference of diagram 11 and 12	

Diagram 1–4, 8–10 illustrate the greatest number of antigens detected by an ideal mixture of the antisera, indicated by our numbers. (S = rat serum, D = protein extract D, R = protein extract R, H = proteins of liver homogenate)

Zusammenfassung. Blutserum-Eiweisse der Ratte und aus Rattenleber isolierte Proteine wurden immunoelektrophoretisch und mit Ouchterlonys Doppeldiffusion untersucht bzw. verglichen. Die Ergebnisse sprechen für Ähnlichkeit oder Identität diverser Serum- und Lebereiweisse.

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What Constitutes 'Apparato Reticolare Interno' of Golgi in the Goblet Cells of Vertebrate Intestine?

CAJAL[1], NASSONOV[2], BOWEN[3], DUTHIE[4], CHODNIK[5], and YOKOCHI[6] are a few of many workers who have described the 'apparato reticolare interno' of Golgi in the supranuclear – or secretogenous – or Golgizones of the vertebrate intestinal goblet cells, and have also attributed a vital rôle to it in mucus synthesis. The findings of these earlier cytologists, concerning the existence and the rôle of the 'Golgi apparatus' in these cells, need reinvestigation, especially in the light of the recent illuminating and thought-provoking communications of NATH[7] and BAKER[8], both of whom have categorically denied its very existence from all the vertebrate cells.

The author has carried out, in detail, cytological studies on the intestinal goblet cells of the fish (*Mystus seenghala*), the frog (*Rana tigrina*), the lizard (*Hemidactylus* sp.), and the house- and white rat, with special attention to the problem of the 'Golgi apparatus', by employing, in addition to rational morphological and various current histochemical techniques, old classical 'Golgi techniques' (AOYAMA and KOLATCHEV) and also their various modifications[9,10]. The author has not consistently observed anything in these cells, which could even remotely be compared with the 'basket-like'- or 'reticular Golgi apparatus', reported to have been observed by earlier workers[1–6]. Instead, in the 'Golgi zones' are observed aggregations of either filamentous mitochondria (early recovery and late discharge phases) or distinct lipid bodies (late recovery

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phases) or of both or of maturing secretion globules (secretory phases). These cytoplasmic organelles sometimes give rise to the structures which can simply be homologized with the 'Golgi apparatus' of earlier workers[1–6]. To the best of author's knowledge no one has so far specifically denied the existence of the 'Golgi apparatus' in the vertebrate intestinal goblet cells.

It is intended to find out whether the reasons already put forward by the leading antagonists[7,8,11–14] for the appearances of such deceptive 'Golgi reticula' in a variety of other animal cells from time to time, and also put forth by the author himself[15] in the exocrine cells of pancreas, hold good in case of the cells under discussion and also whether there is any extra reason hitherto not described.

It appears that, in the vertebrate intestinal goblet cells, 'Golgi apparatus' may be formed due to the following causes:

1. The preliminary fixatives used in the classical methods cause adherence of the already aggregated lipid bodies (especially in the Golgi zone) and so the over impregnation is facilitated.

2. 'Golgi networks' may be formed simply by the indiscriminate deposition of silver and osmium between crowded secretion globules (mucus droplets).

3. The chemicals used in the various classical methods (e.g. cadmium chloride, chromic acid etc.) are protein precipitents and hence these disrupt the lipid bodies (which in these cells are lipoproteinous histochemically) which serve as segregating membranes for the mucus–which also have proteinous moiety[16]. These disrupted and closely apposed lipid sheaths are then heavily impregnated and give the appearance of typical 'Golgi reticula' in these preparations.

4. Filamentous mitochondria, particularly of rats, are appreciably impregnated by osmium and silver. When such heavily impregnated mitochondrial filaments along with still more heavily impregnated and disrupted lipid bodies (*vide supra*) aggregate in large number in the 'Golgi zone', particularly in recovery phases, these lose their individual identity and give the appearance of typical 'Golgi apparatus', constituting of haphazardly dispersed tortuous solid strands.

In addition, there has been observed, after employing various histochemical techniques[17–19], an intense PNA (not RNA; see KANWAR[20]) concentration in the 'Golgi'- and basalzones of the goblet cells. These PNA-rich areas take up intense blue diffuse haematoxylin (particularly in case of the fish) stain (sufficient to obliterate other finer cellular details) in preparations which do not at any stage involve $K_2Cr_2O_7$ treatment ($K_2Cr_2O_7$ dissolves PNA). These PNA-rich areas, which have sufficient diffuse lipids, also reveal intense metallic impregnation in various 'Golgi preparations'. Since the mucus droplets are neither impregnated nor stained with haematoxylin, these appear as hyaline spaces intermingled with intensely stained or heavily impregnated scanty cytoplasmic strands. All this gives the appearance of a typical 'basket-like Golgi apparatus' of BOWEN[3].

More or less similar explanation, holding basophil cytoplasmic strands responsible for such deceptive appearances, has recently been put forward by MALHOTRA[21] who worked on the vertebrate neurones.

The above observations, denying the very existence of the 'Golgi apparatus', are fully supported by TAYLOR[22], who studied the mammalian intestinal goblet cells under electron microscope.

Résumé. L'auteur montre que l'appareil réticulaire classique de Golgi dans les cellules caliciformes intestinales des Vertébrés est un produit artificiel. Diverses raisons sont alléguées pour expliquer ce fait.

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Pure Males and Females from Hermaphroditic Strains of *Ophryotrocha puerilis*

Ophryotrocha puerilis is a proterandrous hermaphroditic Polychete worm[1]. HARTMANN et al.[2] showed that reversal from female to male phase can be obtained in this species by means of various environmental factors, and BACCI[3] demonstrated that the action of such factors has different expression in the Mediterranean and Atlantic subspecies which are named *O. puerilis puerilis* and *O. puerilis siberti* respectively[4].

Selection experiments both for the prolongation of the male and for the anticipation of the female phase[5] were positive in both directions and the minus selection produced at generation some 4 individuals that showed oocytes at a length of 11 or 12 chaetigerous segments, which practically did not undergo a male phase. The plus selection produced (also at generation 4) individuals that reached the length of 25 or 26 segments and died without showing any oocyte during the whole life. Such experiments thus demonstrated the existence of multiple sex genotypes in *Ophryotrocha puerilis*.

Experiments on sex determining mechanisms were resumed in 1959 on a strain of *O. puerilis siberti* from ROSCOFF[6] and they gave results exactly comparable to those obtained in classic research work on polygenes.

The prosecution of selection experiments in the ROSCOFF strain has at present led to the production of pure males in generation 5: two individuals have reached the length of 42 chaetigerous segments (which is the maximum length so far observed in *Ophryotrocha puerilis*) always remaining in the male phase (Fig. d). One of them has been employed in a cross with a female phase individual of 13 segments and the other has been kept isolated and it is now showing clear signs of senility. About 100 individuals of the generation 5 to which the two pure males

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