proportion of soluble collagens in collagen fibre decreases during ageing[18]. This leads to the presumption of the splitting of a soluble collagen containing a higher concentration of hydroxyproline than the remaining insoluble collagen. HALL[19] actually reports that alkali soluble collagen from hide powder contains in a particular fraction an extremely high concentration of hydroxyproline (till 38 weight%). But this is in contradiction with the abovementioned analysis of soluble collagens[2-5].

To elucidate this discrepancy, we extracted all collagens from lung tissue using hot trichloracetic acid and the concentration of proline, and hydroxyproline was referred to the amount of nitrogen in extract. We found that, in lung extracts from various age groups of rats, the proline concentration does not change whereas the concentration of hydroxyproline is more than double during ageing: proline-hydroxyproline ratio amounts in collagens from 9 days old rats to 4.3; in 14 days old rats to 3.7; 30 days old rats to 3.0; 40 days old rats to 2.1; 60 days old rats to 1.6.

This increase in hydroxyproline concentration in collagen during ageing is not inherent to collagens extracted from organs only but to all forms of collagens in maturing rats. In Table II we present the results of analysis of chemically non-modified collagen fibers prepared mechanically from tail tendon of newborn and adult rats. In very young rats, the collagen fibre contains significantly less hydroxyproline than in collagen fibre from adult animals. The concentration of proline was the same in different samples, without regard to the age of the animals.

We suppose, therefore, that in early stages of development of rats the non-mature collagen is formed by a different aminoacid composition than is found in matured collagen. It is not yet clear how the maturation is realized, namely by which means the increase of hydroxyproline in collagen molecule occurs. It is worth mentioning that in peptides containing hydroxyproline isolated from different animal tissues[20], we found similar changes in the ratio of proline to hydroxyproline.

Zusammenfassung. Durch Bestimmung des Prolin- und Hydroxyprolingehaltes in unlösbarem Kollagen und in den gesamten Kollagenen der Lunge von Ratten verschiedenen Alters wurde bewiesen, dass in der frühen postnatalen Epoche der Ratte (bis zu 30 Tagen) die Menge des Hydroxyprolins im Kollagen eindeutig steigt, während sich die Menge des Prolins praktisch nicht ändert.

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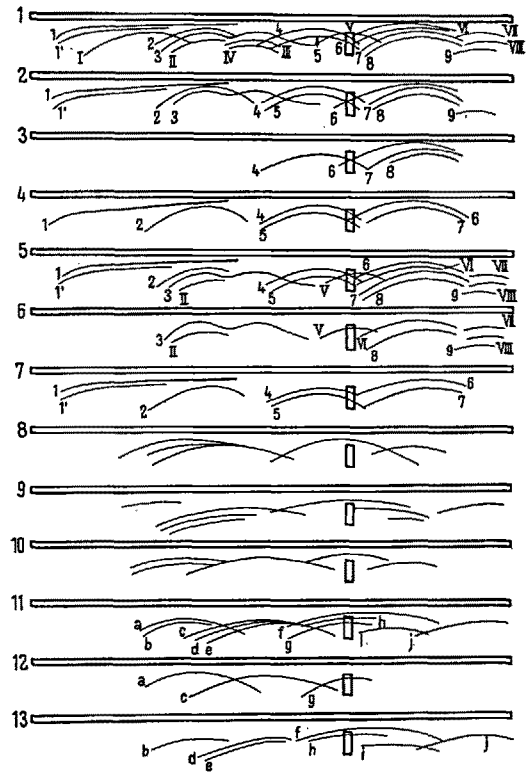
The Relation between some Serum and Liver Proteins in Rats Studied by Immunoelectrophoresis and by Ouchterlony's Double Diffusion Method

We investigated the relation between liver and serum proteins in rats both by immunoelectrophoresis[1,2] and by the double diffusion method in agar[3] with the technique of transferring agar discs with the isolated fractions[4].

The experiments were carried out with serum, liver homogenate and extracts from livers washed *in situ* from adult white rats. The extract marked R was obtained by centrifuging 30% of the homogenate of washed rat livers in a phosphate buffer (0.1 m pH 7.5) in a cooled laboratory centrifuge at 5000 g. Particles difficult to centrifuge were easily separated by the procedure quoted in one of our previous papers[5] after the addition of $^1/_3$ of the total volume of a rivanol (2-etoxy-6, 9-diaminoacridin lactate) solution. After centrifugation, the rivanol present in the supernatant was separated by absorption on active coal (Norit SX 30). The second extract analyzed, marked D, was obtained by centrifuging the 30% homogenate of washed rat livers with saline; particles difficult to centrifuge were precipitated with the help of dextran-sulphate in the presence of calcium chloride. The method was analogous to that used for the isolation of serum lipoproteins according to BURSTEIN[6]. Both extracts were dialyzed and lyophilized. All the work was performed at a temperature of 0–3°C.

The antisera used were obtained by immunizing 4 groups of rabbits for 5 weeks (each group consisting of 3–4 rabbits) with the following antigens: rat serum, 10% homogenate of washed rat livers in saline, extracts R and D (the solution of both extracts contained about 7% proteins).

The results of the immunoelectrophoretic analysis of the serum are given in the diagrams 1–4 (Fig.). On using specific antisera, we succeeded in detecting up to 17 precipitation lines. These lines were numbered with Arabic and Roman numerals. Arabic numerals were used



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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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