Lipoproteins that are included in serum have a close relation to metabolism of lipid, and they are noticed in the research field on hyperlipemia. This time, lipoproteins were separated gradually from human serum with the preparative micro ultracentrifuge and the 6PC thick tubes (actual capacity: 5.3 ml). It is possible to carry out centrifugation under the conditions similar to those of the large ultracentrifuge because serum can be centrifuged up to 3.4 ml per tube in the S80AT3 angle rotor. The specific gravity liquid was prepared according to the reference.

1. Instruments used
Centrifuge: Hitachi CS-GX preparative micro ultracentrifuge
Rotor: S80AT3 angle rotor
Tube: 6PC thick tubes

2. Procedures and results
1) Separation of VLDL, including chioromicron (\( \rho <1.006 \text{ g/cm}^3 \))

![Diagram](https://via.placeholder.com/150)

- Sample 3.4 ml
- Add 1.7 ml of specific gravity liquid A on the layer.
- Centrifugation: 80,000 rpm
- 4.5 hours, 16°C
- ACCEL: 5
- DECEL: 7

Upper layer: VLDL

Remove 1.7 ml of the VLDL layer (upper layer) of 1). Transfer 3.4 ml of the lower layer to another tube.

2) Separation of LDL, including IDL (1.006 g/cm³<\( \rho <1.063 \text{ g/cm}^3 \))

![Diagram](https://via.placeholder.com/150)

- Add 1.7 ml of specific gravity liquid B and mix well gently.
- Centrifugation: 80,000 rpm
- 5.5 hours, 16°C
- ACCEL: 9
- DECEL: 7

Upper layer: LDL (including IDL)
3) Separation of HDL (1.063 g/cm³ < ρ < 1.21 g/cm³)

Remove 1.7 ml of the LDL layer (upper layer) of 2). Transfer 3.4 ml of the lower layer to another tube.

Add 1.7 ml of specific gravity liquid C and mix well gently.

Centrifugation
80,000 rpm
8 hours, 16˚C
ACCEL: 9
DECEL: 7

Upper layer: HDL
Lower layer: albumin, etc.

Specific gravity liquid A*: 1.006 g/cm³
Specific gravity liquid B*: 1.182 g/cm³
Specific gravity liquid C*: 1.478 g/cm³

Fig. 1 Result of electrophoresis*

VLDL → LDL → HDL

Direction of electrophoresis

1. Serum before centrifugation
2. VLDL fraction
3. LDL fraction
4. HDL fraction

*1: Specific gravity liquid A:
Put 11.40 g of NaCl and 0.1g of EDTA-Na2 in a 1,000 ml measuring flask. Add 500 ml of distilled water and 1 ml of 1N NaOH, and mix them well until they are dissolved. Add distilled water up to 1,000 ml and then add additional 3 ml of distilled water. (NaCl: 0.195 mol)

*2: Specific gravity liquid B:
Add 24.98 g of NaBr to 100 ml of the specific gravity liquid A. (NaCl: 0.195 mol, NaBr: 2.44 mol)

*3: Specific gravity liquid C:
Add 78.32 g of NaBr to 100 ml of the specific gravity liquid A. (NaCl: 0.195 mol, NaBr: 7.65 mol)

*4: PAG disk electrophoresis for lipoprotein: Lipophor kit (JOKOH CO., LTD.)

For further information, please contact Hitachi Koki Scientific Instruments Group.