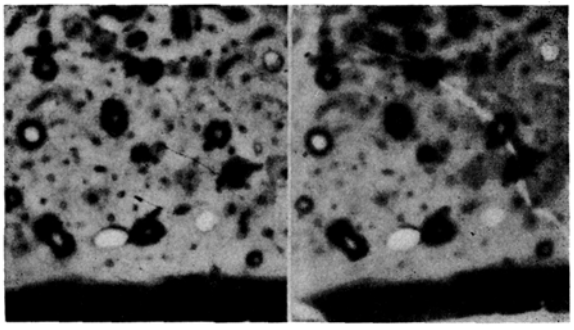
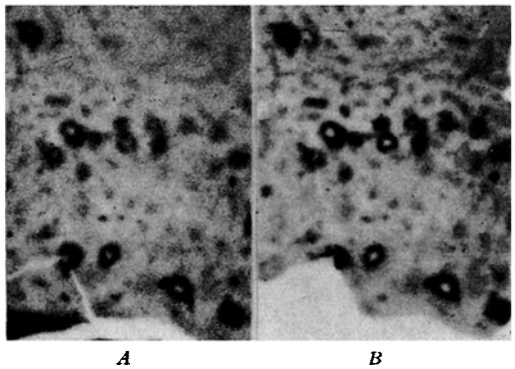
sections of total bone treated with radiocalcium are partially decalcified (or incinerated and decalcified) to remove from 1/10 to 1/8 circa of their mineral content. The total radioactivity of the section is thus greatly reduced, but in a rather uniform degree in the various structures (Fig. 4.)



A *B*

Fig. 3.–Calf, metatars. *A*, section of total bone treated with Ca^{45} for 18 days; *B*, the same after ashing (GABRIEL). Autographs 16 ×.



A *B*

Fig. 4.–Calf, metatars. *A*, section of total bone treated with Ca^{45} then ashed; *B*, the same after partial decalcification. Exposure times of autographs 1:3. 13 ×.

(6) Autographs made from strips of periosteum peeled off from bone compacta (fixed in ethanol) and treated with radiocalcium show radioactivity only in limited areas which correspond to small spicules of bone adherent to the fibrous tissue. R. AMPRINO

Institute of Anatomy, University of Turin, July 15, 1952.

Résumé

L'auteur s'est proposé de préciser les conditions qui règlent les différences quantitatives de fixation du radiocalcium dans la structure des os à différents stades de formation. De minces lamelles d'os usées et polies ont été soumises *in vitro* à des traitements divers (microincinération à 500° ou à 700°C, gabriélisation, décalcification partielle, traitement par la hyaluronidase, par l'acide phosphowolframique, etc.) et traitées en suite avec une faible solution de chlorure de Ca^{45}. L'étude des autoradiographies démontre que seule la destruction totale des composants organiques de la matrice osseuse entraîne des modifications appréciables de la distribution du radiocalcium.

Researches on the Chemical Composition of the Erythrocyte Membrane

At present the chemical composition of the erythrocyte membrane is still little known. Our researches are intended to contribute further data and clarity on the protein composition of the normal erythrocyte membrane, and at a future stage to see if any deviation from the normal exists in anemiae due to haemolysis and erythrocyte fragmentation.

From erythrocyte shadows, precipitated from 50 to 100 cc of blood with the CO_2 method, stromatin (a fibrous protein) was extracted with EDSALL-WEBER's liquid[1]. This extracted substance was precipitated several times to remove all the possible Hb present. All the aforesaid operations took place at low temperature. The precipitate was again dissolved in EDSALL-WEBER's liquid and chromatographed on WHATMAN's No. 1 filter paper using pyridine (80) + water (20) as solvent: ascensional method, time employed 12–18 h.

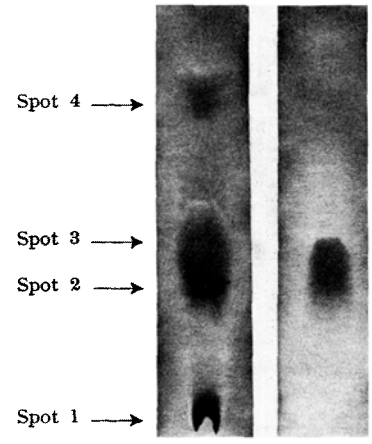


Fig. 1.

The Iodine evidence showed up 4 different spots (Fig. 1*A*) besides the frontal one which was partly due to the impurities of solvents, paper and partly due to traces of Hb.

Figure 1*B* represents the chromatogram of the top liquid after the first precipitation operation. It shows a spot corresponding to a substance which was not submitted to further researches.

In order to obtain and study a certain quantity of substances of which the 4 spots are made up, we chromatographed the substance by placing it on the paper along a horizontal line at a distance of about 3 cm from the edge immersed in the solvent. After drying the paper 3 strips were cut parallel to the course followed by the liquid (2 at the lateral edges and 1 central); the test was made with Iodine (Fig. 2).

Using these strips as guides we cut the strips corresponding to the 4 spots. The substances were eluated with EDSALL-WEBER's liquid according to CONDSEN, GORDAN, MARTIN's method[2]. After dialysis through cellophan, the 4 substances were hydrolized for 8–10 h. with 20% HCl and after removing the HCl, the hydrolizate was

[1] M. CIGADA, P. CITTERIO, A. ORLANDI, S. RANZI, and L. TOSI, Rend. Istit. Lombar. Sci. Lett. (Cl. Scienze) *82*, 351 (1949).

[2] R. CONSDEN, A. H. GORDON, and A. J. P. MARTIN, Biochem. J. *41*, 590 (1947).

bidimensionally chromatographed on WHATMAN's No. 1 paper: 1st liquid = n-Butanol + ac. acetic + H_2O; 2nd liquid = phenol + H_2O and NH_3 + KCN.

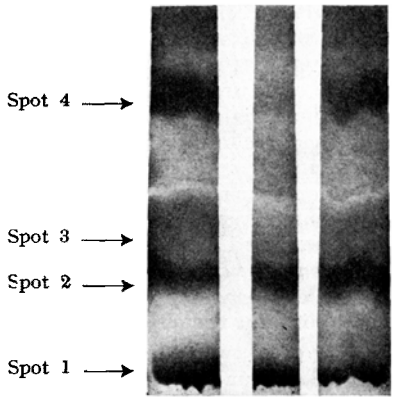


Fig. 2.

After spraying with ninhydrin the 4 hydrolized spots showed an almost identical chromatogram: 13 spots were stained with ninhydrin (amino acids) and 3 large spots were unstained (Fig. 3).

Fig. 3.

In the belief that the afore-mentioned 3 unstained spots may also be represented by lipids, strips corresponding to the 4 spot-substances were extracted in Kumagawa with ether and alcohol-ether.

The substances extracted showed some organoleptic qualities of the lipids. The chemical findings are presented in the Table.

These findings seem to demonstrate that the 4 spots are made up of substances which are probably fibrous lipo-proteins. Now arises the question whether the 4 substances isolated correspond either to 4 different proteins or to 4 fragments of the same protein or merely artefacts due to the technique used.

	HÜHL's Test	LIEBERMANN's Test	Nitro-Chromic Test[1]	P Test
Spot No. 1 . .	+	+	+	+
Spot No. 2 . .	+	+	–	–
Spot No. 3 . .	+	+	+	+
Spot No. 4 . .	+	+	–	–

The reproducable experiment can discount the possibility of an artefact. Further proof against an artefact is that three times during the paper chromatogram preparations the test tube containing the solution of the substance extracted with EDSALL-WEBER's liquid was kept at room temperature instead of in ice: in each case we saw, instead of 4 isolated spots, a single spot distributed along the whole course of the liquid as though the change of temperature had brought about a denaturation of the 4 substances.

This fact, together with the chemical analysis of the lipoid portions, which shows that the 4 lipoid parts are not alike, speaks in favour of the hypothesis of the existence of 4 different proteins.

The 4 protein fragment hypothesis, on the other hand, may be deduced by analogies made by BAILEY[2] concerning tropomyosin.

To support the 4 protein hypothesis and to confirm our findings, other researches elsewhere have given proof that, besides the existence of Hb, the stromatin is not the only protein found in the red blood cells. STERN and co-workers[3] isolated by electrophoresis from the stroma residue an "a-protein" which is probably stromatin and a "b-protein" which is as yet unidentified. CALVIN and co-workers[4] separated another protein from stromatin called elinin, which could be divided by electrophoresis into two components: a main component (80%) and a faster component (20%). The aformentioned authors were unable to say if stromatin and elinin were two different proteins or if they were simply two fractions of the same protein.

Although our research findings and those of the authors hereby cited are not completely identical, the fact remains that the ultimate results of each group lead to the conclusion that the red blood cells contain either several fibrous lipoproteins or a single protein which breaks up in two or more fragments.

L. PEROSA and G. RACCUGLIA

Institute of Clinical Medicine, University, Bari, Italy, March 18, 1952.

Riassunto

Gli autori, con un metodo del tutto originale e applicabile a relativamente piccole quantità di sangue (50–100 cm^3) credono di essere riusciti ad isolare 4 lipoproteine dallo stroma eritrocitario.

[1] The nitro-chromic reaction for revealing the presence of primary and secondary alcohol groups.

[2] K. BAILEY, Brit. Med. Bull. *5*, 338 (1948).

[3] K. G. STERN, M. REINER, and R. H. SILBER, quoted by E. PONDER: *Hemolysis and related phenomena* (Grune & Straton, New York, 1948).

[4] M. CALVIN, R. S. EVANS, V. BEHRENDT, and G. CALVIN: quoted by E. PONDER: *Hemolysis and related phenomena* (Grune & Straton, New York, 1948).