TABLE I. Measurement of Rates of Glyceride Absorption.

No. of rats	Glyceride* and supplements	Amt fat fed, mg/dm²	% lipid recovered from		Lipid absorbed†		
			Stomach	Intestine	Abs. amt, mg/dm²/hr	% of fed	% of avail.
8	TG with bile and albumin	90.4	20	13	20.1 ± 3.4	67 ± 11	84 ± 4
8	MG emulsified with bile and albumin	103.7	44	6	16.1 ± 5.3	50 ± 11	89 ± 4
9	TG emulsified with bile and 1/5 MG	135.6	41	9	22.9 ± 7.9	51 ± 17	85 ± 6
11	MG emulsified with bile	160.3	60	6	16.2 ± 8.6	34 ± 15	85 ± 6

* MG and TG represent glyceryl monooleate and triglyceride (olive oil), respectively. The former was supplied by Distillation Products Industries.
† Including stand. dev.

These findings do not imply that both the indirect and direct methods as they are now used are not valid measurements of rate of fat absorption when gastric retention does not occur. However, the more consistent results, obtained when considering only the fat which is readily available for absorption, do suggest that a greater gastric retention in a few of a series of animals may aid in accounting for some of the variations in results. Hence separate recoveries of unabsorbed lipid from both gastric and intestinal contents should give more exact information about the absorptive process.

Summary. Gastric retention of a lipid may lead to erroneous conclusions when rates of fat absorption are measured by the generally accepted methods. Separate recoveries of the remaining lipids from the stomach and intestines after an absorptive period permits a correction for the lipid unavailable for absorption in relative rate calculations.

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Isolation of a Sulfated Mucopolysaccharide from Blood Platelets of Rats.* (22883)

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It has been observed(1) that blood platelets of rats incorporate S^{35}-labeled sulfate; and cytochemical evidence indicates that platelets contain mucopolysaccharides(2). Since it is known that sulfate becomes incorporated into mucopolysaccharides(3-5) but is not incorporated(6,7), or incorporated in only negligible amounts(8), into sulfur-containing amino acids of rats, we undertook this study to determine whether the S^{35}-sulfate in rat platelets is present in a mucopolysaccharide.

Materials and methods. Male Sprague-Dawley rats, 9-11 months old and weighing 410-538 g, were used as platelet donors. The platelets were labeled by injecting each rat intraperitoneally with 1 mc of $S^{35}O_4^{--}$ daily

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for 3 days.† Forty-eight hours after the last sulfate injection and 10 minutes after intravenous injection of 0.5 ml of a 1:10 dilution of heparin, the blood was drawn from the abdominal aorta into a siliconed syringe containing 0.5 ml of a 1% solution of Sequestrene‡ in 0.7% saline. The platelets were separated from the blood by differential centrifugation by a modification of the method of Dillard *et al.*(9). After the blood was centrifuged at 45 X *g* for 60 minutes at 5°C, the platelet-rich plasma layer was withdrawn and centrifuged at 500 X *g* for 30 minutes (5°C) to obtain the platelets. The platelets were then washed 3 times with 10-ml portions of saline and resuspended in saline to a concentration of 3.575 x 10^6 to 4.86 x 10^6 platelets/mm^3. One-ml portions of the platelet suspensions were lyophilized and stored in a refrigerator until extracted.

The sulfate-labeled platelets were extracted with 0.5 N NaOH for 7 hours at 0°C with constant stirring [modification of the method of Jorpes(10)]. The thoroughly suspended mixture was sampled for radioactivity measurement and spun at 25,000 X *g* for 15 minutes. The supernatant was sampled for radioactivity, neutralized immediately with 2 N acetic acid (dibromothymolsulfonphthalein was used as the indicator), and treated with chloroform-amyl alcohol [Sevag technic(11)] for removing protein until only a slight precipitate formed (15 to 20 times). Samples of the aqueous supernatant were taken frequently during this procedure for radioactivity measurement. The aqueous supernatant was dialyzed for 40 hours against four 1000-ml portions of distilled water, and samples were taken for radioactivity measurement. The dialyzed material was lyophilized to reduce volume and for keeping. For electrophoresis or chromatography the lyophilized material was dissolved in a small amount of 0.1 M phosphate buffer with a pH of 6.6.

Electrophoresis was carried out in a Spinco cell, Model R-Series C, Durrum type, by a method similar to that of Rienits(12). Strips of Whatman #2 filter paper 3.0 X 30.5 cm were run with 0.1 M phosphate buffer at pH 6.6 as the electrolyte for 3 hours at 10.8 V/cm (0°C). The strips were dried in an oven at 115°C, stained 30 minutes in 0.1% Alcian blue 8 GX 300§ at pH 2.5, and were washed in several changes of N/5 acetic acid and finally with water. As little as 1 μg of commercial chondroitin sulfate can be detected on Whatman #1 filter paper by this method.

Paper chromatography was carried out by a modification of the procedure of Kerby(13). Samples were applied a little at a time to sheets of Whatman #1 filter paper (15.5 by 11.25 inches), the spot being dried with a hair dryer between applications. Each spot of labeled platelet extract was measured for S^{35} activity. Each paper was then stapled together to form a cylinder and was run in an individual jar at 4°C for 32 hours, the solvent being 100 ml of a 35% solution of propanol in M/15 phosphate buffer having a pH of 6.4. The chromatograms were dried at room temperature and stained with Alcian blue.

Water lysates were prepared from platelets similarly labeled but harvested 24 instead of 48 hours after the last sulfate injection. The washed packed platelets were extracted 6 times with 1 ml of distilled water. Each mixture was spun at 1800 X *g* for 15 minutes to obtain a clear supernatant, and all supernatants were combined after the final extraction. One portion of a water lysate was treated with chloroform-amyl alcohol until only a small precipitate formed (15 times).

Samples for measurement of radioactivity were placed on flat steel planchets, air dried, and counted in an internal gas flow counter.

† $S^{35}O_4$ in weak HCl solution received from the Isotopes Division, Oak Ridge National Laboratory, was neutralized with NaOH (dibromothymolsulfonphthalein indicator).

‡ The Sequestrene (disodium ethylenediamine tetraacetic acid) was kindly supplied by the Alrose Chemical Co., Providence 1, R. I.

§ The Alcian Blue 8 GX 300 contains the same colored entity as Alcian Blue 8 GN used by Rizzoli and Gliozzi(14) and as Alcian Blue 8 GN 150, which we have also used successfully. Alcian blue 8 GX 300 and Alcian blue 8 GN 150 were kindly supplied by Arnold, Hoffman and Co., Inc., Providence 1.

TABLE I. Extraction of Radioactivity from $S^{35}O_4$-Labeled Platelets Treated with .5 N NaOH.

Extract No.	Extract mixture,* c/m/50 μl	Supernatant,† c/m/50 μl	% recovery after Extraction	Protein Pptn.	Dialysis
III	1452	1246	86	—	61
IV	2068	2096	101	100	—
V	2308	2273	98	100	45

* Comparable to whole platelets.
† After centrifuging at 25000 × *g* for 15 min.

Paper electrophoresis strips were cut into pieces 3 to 10 mm wide, placed on planchets, and counted in the same instrument. Chromatograms were counted with a large GM tube that was covered with Mylar film and gassed with Q gas.

Autoradiograms were made of some chromatograms and paper electrophoresis strips by exposing them to Kodak No-Screen X-ray safety film.

Results. From 86 to 100% of the S^{35} was extracted from the lyophilized platelets by 0.5 NaOH, and the activity remained in the aqueous fraction during the Sevag procedure for removal of protein (Table I). When the material extracted from platelets with distilled water was subjected to the Sevag technic, radioactivity was removed in proportion to removal of protein (Table II).

During dialysis of the alkali extract about 50% of the activity was lost (Table I). The S^{35}-labeled dialyzate when subjected to analysis by paper electrophoresis was found to migrate to the same place as chondroitin sulfate (CSA) and heparin or immediately behind these compounds (Fig. 1). In a mixture of CSA with the alkali extract of platelets, the labeled material and CSA migrated to the same place (CSA located by staining and the unknown by counting radioactivity).

The unknown compound was stained with Alcian blue, a dye considered to be specific for mucopolysaccharides(14).

TABLE II. Radioactivity of Supernatants during Deproteination of Distilled Water Lysates of Platelets by Chloroform-amyl Alcohol Treatment (Sevag).

No. of treatments	Radioactivity of supernatant, c/m/200 μl	% of original activity
0	3060	100
8	934	31
15	351	11

One dimensional chromatograms of the unknown compound run by a method(13) which distinguishes between CSA and heparin revealed that its mobility was similar to that of CSA but unlike that of heparin (Fig. 2).

When lyophilized platelets were refluxed with ethyl alcohol in a Soxhlet apparatus for 24 hours to remove lipids, only 5% of the radioactivity originally present in the platelets was recovered in the extract.

Discussion. It is evident that the S^{35}-labaled compound in platelets is not an alcohol-soluble lipid. In its unhydrolyzed condition, as extracted from platelets with distilled water, it appears to be associated with a protein because the radioactivity is removed in proportion to the removal of protein; however, when the platelets are extracted with alkali, an S^{35}-labeled nondialyzable compound can be separated from the protein. This suggests that the sulfur is present in a mucopolysaccharide, since alkaline extraction is a method commonly used for obtaining mucopolysaccharides from tissues(10) (*e.g.*, CSA from cartilage). In addition, after vigorous hydrolysis of platelets with HCl, radioactivity was found, upon precipitation with $BaCl_2$ (Na_2SO_4 added as carrier), to be present in $BaSO_4$ but was not present in lead sulfide when this compound was precipitated (cystine added as carrier), indicating that the activity was not present as amino acid sulfur. Furthermore, the sulfur-containing platelet material stains with a dye considered to be specific for mucopolysaccharides(14), and its migration upon paper electrophoresis is similar to that of chondroitin sulfate and heparin. Finally, when subjected to paper chromatography with a technic that separates

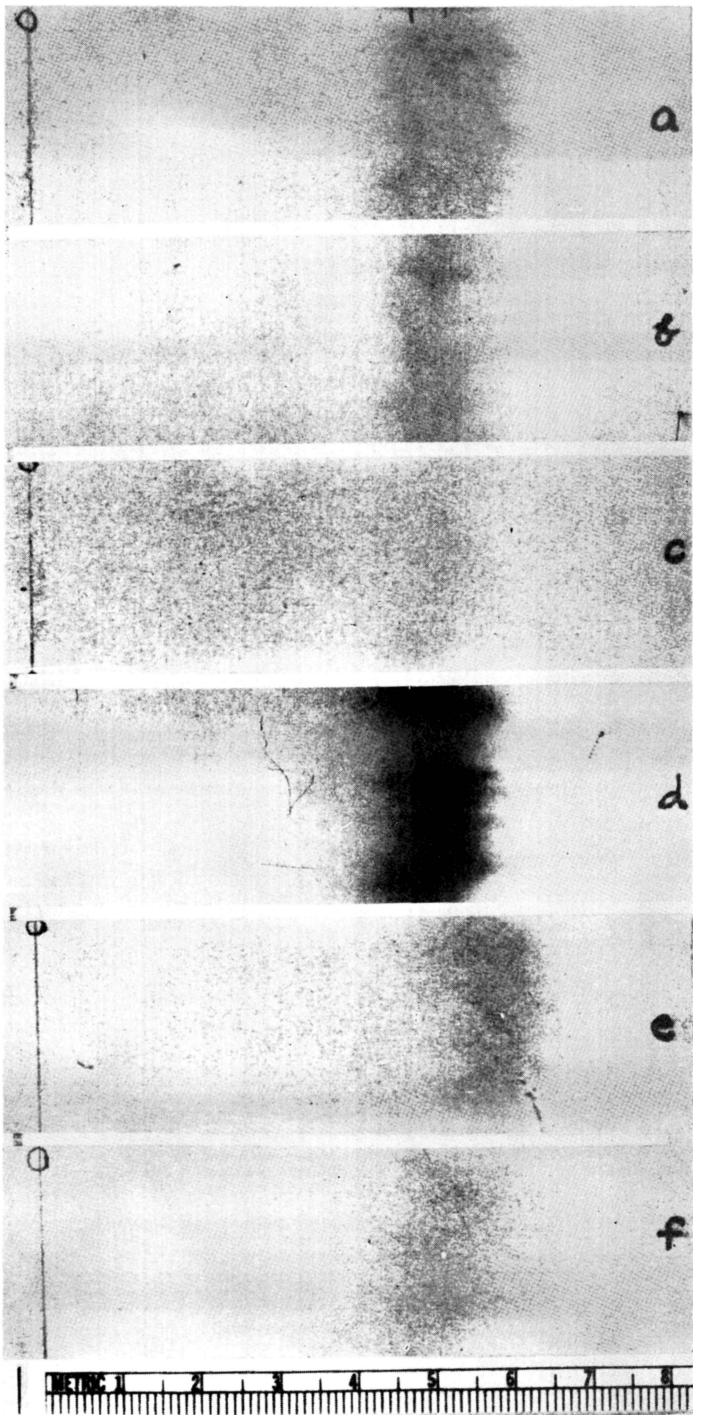

FIG. 1. Paper electrophoresis; line marked O indicates the origin. a, chondroitin sulfate plus platelet extract (non-dialyzable fraction); b, autoradiogram of a; c, platelet extract (non-dialyzable fraction); d, autoradiogram of c; e, chondroitin sulfate; f, heparin.

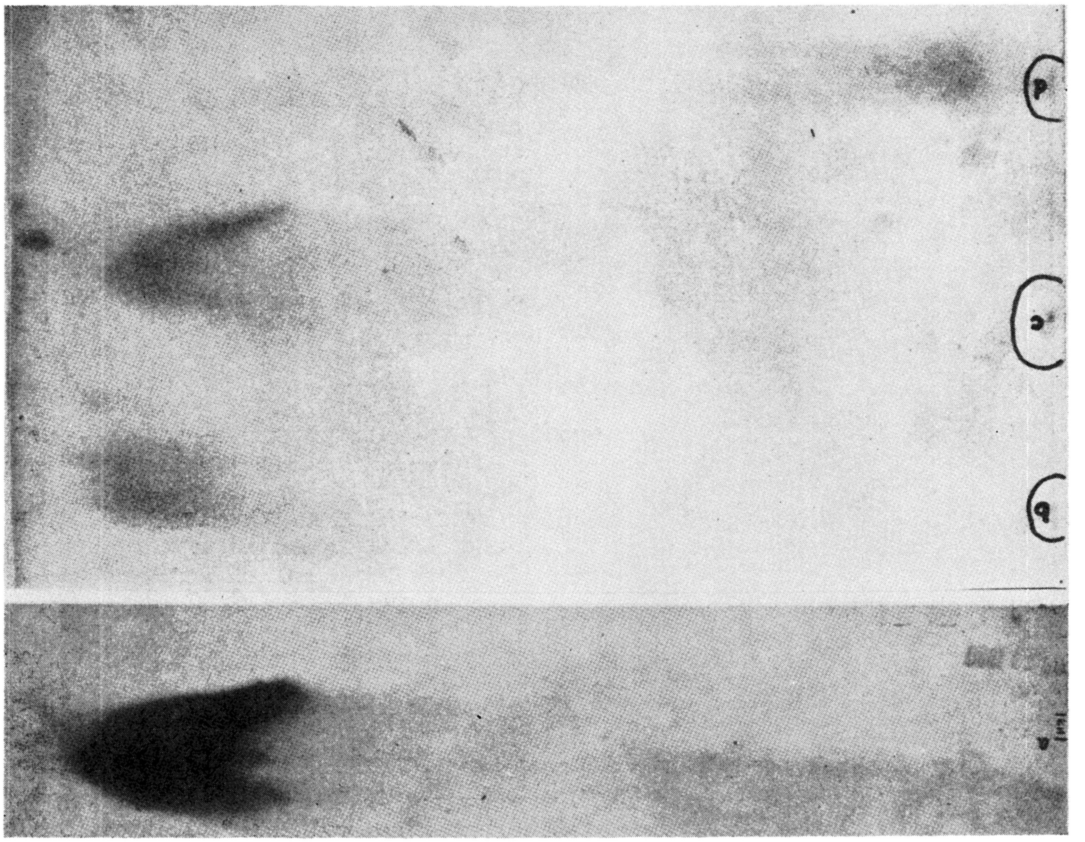

FIG. 2. Paper chromatogram; circles represent origin of the spots. a, autoradiogram of the platelet extract (non-dialyzable fraction); b, chondroitin sulfate; c, platelet extract (non-dialyzable fraction); d, heparin.

chondroitin sulfate and heparin(13), it behaves like chondroitin sulfate. However, its true identity awaits further chemical characterization.

Loss of about 50% of the radioactivity during dialysis has not been explained. The dialysis water was not analyzed by paper chromatography or by paper electrophoresis, but in one experiment it was combined, evaporated, filtered, and carrier sulfate was added. $BaSO_4$ was then precipitated by addition of $BaCl_2$. Counting revealed that 66% of the radioactivity which dialyzed out was recovered in the $BaSO_4$. This barium precipitate may be inorganic sulfate or a product of the partial breakdown of the sulfur-containing compound during alkali extraction.

Care was taken during separation of platelets from the blood to minimize contamination with white blood cells, since a mucopolysaccharide has been found in human leukocytes(15,16). In many platelet suspensions prepared as in these experiments the white blood cells have been counted and the platelet volume has been estimated to be more than 100 times that of the white cells. Hence it is not likely that the sulfate-containing material present in the platelet extracts resulted from contamination by leukocytes. Furthermore a mucopolysaccharide, removable with testicular hyaluronidase, has been demonstrated cytochemically within individual platelets separated by the technic used in this study (2).

It has been suggested that platelets may contribute to the maintenance of the interendothelial cement of the capillary walls(17), possibly by way of a platelet mucopolysaccharide. Another possibility is that the sulfonated mucopolysaccharide described here

may have either thromboplastic or antithromboplastic activity; it has been reported that hyaluronic acid has thromboplastic activity (18), whereas sulfonation of hyaluronic acid produces a strong heparin-like anticoagulant(19).

Summary. Most of the radiosulfate in S^{35}-labeled rat blood platelets was recovered in an alkali extract. The extracted material stained with Alcian blue for mucopolysaccharides and was found to behave similarly to chondroitin sulfate when subjected to paper electrophoresis and paper chromatography.

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Effect of 2-Methyl-9 Alpha Fluorohydrocortisone on Internal Distribution of Fluid and Electrolytes of Fasted Adrenalectomized Dogs.* (22884)

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It has been suggested(1-3), that animals lacking adrenal cortical hormones are unable to dilute their blood by mobilizing the water and salt in cells and tissues for transfer to the vascular system to maintain a volume of circulating fluid compatible with life. These early experiments indicated a regulatory influence of the adrenal cortex over the internal distribution of water and certain electrolytes. More recently several investigators(4-8), using DCA, cortisone and ACTH, have obtained convincing evidence for steroid induced water and electrolyte shifts between intra and extracellular compartments. The following experiments in which the potent corticoid 2-methyl-9α-fluorohydrocortisone (2-methyl FF), was employed, seem to afford unequivocal evidence that adrenalectomized dogs, exhibiting severe symptoms of insufficiency, even though deprived of food and water possess sufficient water and salt reserves in their cells and tissues to completely restore normal activity and vigor. However, they are unable to mobilize and redistribute these substances in the absence of adrenal hormones. When potent corticoids are administered i.v., water and electrolytes are withdrawn from tissues and cells in amounts adequate to (1) fully restore the failing circulation; (2) restore the normal electrolyte pattern of the blood; (3) induce renal elimination of the accumulations of K and blood urea nitrogen and (4) reduce the hemocon-

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