contains definitely less enzyme than the cortex and the pattern of distribution is like that in the lower animals.

In this region below the cortex, which in the primates shows the increased enzyme content, are found fibers which make extrinsic cortico-cortical connections. The coincidence of this pattern of distribution with the development in the primates of the capacity for wider mental association, as found in man, may be of significance. If this difference in the pattern of distribution proves to be tenable after further study, it would differentiate qualitatively between the brain of primates and that of the lower animals.

Summary. Within the pallium a quantitative distribution pattern of carbonic anhydrase, not found in the dog, cat, hog, horse, sheep, cattle and rabbit, has been found in man and the Rhesus monkey. Its possible significance is discussed.

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Purification of the Resin Amberlite IR-100 for Blood Coagulation Studies.*

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The phenol-formaldehyde resin, Amberlite IR-100, which can remove calcium from blood completely by virtue of its ion exchange property[1] has become a valuable agent for quantitative studies of calcium in the coagulation mechanism.[2,3] Since certain lots of Amberlite have been found to contain impurities which interfere with coagulation, an improved method of purification had to be devised. Several studies have been carried out with the purified product to determine further whether Amberlite induces any change in blood other than the removal of calcium.

Experimental. 1. *Purification of Amberlite.*‡ Twenty grams of Amberlite were covered with 100 cc of 5% (by volume) sulfuric acid and the mixture boiled and stirred vigorously for 5 minutes. The resin was washed with distilled water by decantation until the wash water was no longer acid. The material was then treated with 100 cc of 5% sodium carbonate solution, heated to approximately 70°C and thoroughly stirred. The deeply colored supernatant fluid was poured off and the process repeated until little coloring matter could be extracted. The resin was finally washed with warm distilled water until all traces of sodium carbonate were removed.

The cation-exchange resin was prepared for reaction by adding to it 250 cc of 5% solution of sodium chloride, stirring vigorously for 30 minutes and allowing the mixture to stand for 60 minutes more. The resin was then washed with distilled water and filtered by suction until the wash water no longer contained chloride ions. The solid was dried at 37°C.

2. *Decalcification with Amberlite.* To prevent incipient coagulation, blood obtained by venipuncture with minimal trauma was drawn into a syringe coated with Silicone and immediately passed through a column of 3 g of Amberlite, an amount sufficient for complete decalcification of 10 cc of blood. The

* This work was supported by a grant from the United States Public Health Service.

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1 Steinberg, A., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 124.

2 Quick, A. J., *Am. J. Physiol.*, 1947, **148**, 211.

3 Stefanini, M., and Quick, A. J., *Am. J. Physiol.*, in press.

‡ Mr. James C. Winters of the Resinous Products and Chemical Company offered valuable suggestions.

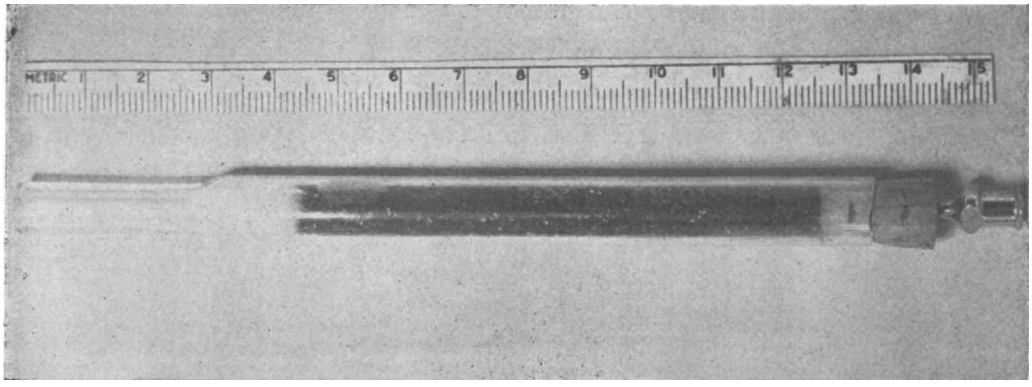

FIG. 1.
Tube Employed for Decalcifying Blood with Amberlite. The tube, the plug of glass wool, and the needle (No. 16) were all coated with Silicone; the cork with collodion.

tube used was approximately 1 x 15 cm and was coated with Silicone§ (Fig. 1). The decalcified blood was collected in a test tube coated with Silicone, then passed twice more through the same Amberlite. This resin can be re-used several times if it is washed free of all traces of blood with distilled water immediately after each use, then treated with 5% sodium carbonate and recharged with sodium chloride.[1]

Properties of the blood decalcified with Amberlite. Steinberg's original findings[1] that the blood was little altered, except by removal of calcium, were confirmed. If any calcium remained, the amount was too small to be determined by ordinary analytical methods. Since dog blood thus decalcified failed to clot after the addition of thromboplastin, the calcium remaining must have been considerably less than 0.0001 M, a concentration still adequate to produce rapid clotting.[2] The thromboplastin used in this experiment and in all of our determinations of prothrombin time of plasma decalcified with Amberlite was made calcium-free. This was done by careful trituration of the rabbit brain from which it was prepared, with 0.02 cc of a 0.1 M solution of sodium oxalate per g of fresh weight, before dehydration with acetone.

Curiously, the sedimentation rates determined with blood decalcified with Amberlite were consistently lower than with citrated or heparinized blood (Table I), thus confirming the findings of Steinberg. This observation may ultimately help to explain the sedimentation behavior of blood.

The clotting time of whole blood decalcified with Amberlite and recalcified with an optimum concentration of $CaCl_2$ (0.00386 M) appears to be much shorter than that of the original blood. For example, the clotting time of 8 minutes 15 seconds for a sample of native human blood was reduced by treatment with Amberlite and recalcification to 3 minutes and 30 seconds. However, a similar result is obtained with blood recalcified after oxalation or citration.

As shown by Steinberg, little or no modification of the prothrombin activity, as measured with the one stage method, takes place in blood decalcified with Amberlite; the fibrinogen content is slightly decreased (10-15%) but not sufficiently to cause any change in the coagulability. Human plasma that has been decalcified with Amberlite after the removal of prothrombin and fibrinogen can restore to normal the apparently delayed prothrombin time of stored human oxalated plasma.

The antithrombin activity of Amberlite plasma was compared with that of citrated or oxalated plasma by two methods. In the first, thrombin of decreasing strength was added to a constant amount of oxalated or Amberlite plasma according to the method

§ Methyl-chloro-silane; Dri Film No. 9987 (General Electric).

TABLE I.
Sedimentation Rate of Citrated, Heparinized, and Amberlite Human and Dog Blood After 1 and 24 Hours.
Westergren's technic was used. Values given are in mm.

Subject	Human blood				Dog blood			
	1	2	3	4	1	2	3	4
After 1 hr								
Citrated blood	10	14.5	23	17.5	2	2	2.5	3
Heparinized "	9.7	15	24	18	2	2	3	3
Amberlite "	4	9	15	10.5	1	1	1	2
After 24 hr								
Citrated blood	57	73	99	91	11	15	22	24.5
Heparinized "	58	75	102	94	10	16	20.5	26
Amberlite "	49	64	71	62	6	10	13	14.5

TABLE II.
Antithrombin Activity of Oxalated and Amberlite Plasmas of Man, Dog, and Rabbit.
Each figure in the table represents the average clotting time in seconds obtained in several experiments.*

Dilution of thrombin		Full strength	½	¼	⅛	1/16	1/32
Human plasma	Amberlite	3	4	6	9	15.5	27
	Oxalated	3	4	6.5	9.5	17	29
Dog "	Amberlite	3	4	6.5	12	17.5	32
	Oxalated	3	4	7	11.5	19.5	35
Rabbit "	Amberlite	3.5	4.5	7	12.5	20.5	38
	Oxalated	3.5	5	7.5	13	22	39

* 0.1 cc of thrombin was added to 0.2 cc of the plasma. The tubes were incubated in a water bath at 37°C. The thrombin was always prepared from human oxalated plasma and stored for an hour or longer, until its activity became constant. It was diluted as required with distilled water.

of Quick.[4] In the second, an equal volume of Quick's "full strength" human thrombin[5] was incubated with Amberlite, oxalated or citrated plasma and the rate of decrease in thrombin activity was measured using normal oxalated plasma as a source of fibrinogen. Details are appended to the tabulated data (in Tables II and III).

The results recorded in Table II show plainly that the clotting response of Amberlite plasma to varying concentrations of thrombin is the same as that of oxalated plasma. Therefore, the Amberlite does not introduce or reduce any agent antagonistic to thrombin, nor does it affect the stability of fibrinogen. When thrombin is incubated with Amberlite plasma (Table III), its inactivation is less pronounced than when it is incubated with oxalated plasma, but essentially the same as with citrated plasma. Further investigation is required to provide an explanation.

Summary. 1. A method of purification of resin Amberlite IR-100 was devised. When blood was treated with the purified resin a decreased sedimentation rate was found, with morphological, chemical, and physical properties otherwise practically unmodified.

2. Of the factors involved in blood coagulation, prothrombin, fibrinogen and the labile factor described by Quick[6] apparently were not appreciably altered by the decalcification with Amberlite. With optimal recalcification, the clotting time of the treated blood was much shorter than that of native blood, but this is also true for oxalated and citrated blood.

3. A reduced antithrombin activity was observed in Amberlite plasma upon incubation with "full strength" thrombin. Since

[4] Quick, A. J., *The Hemorrhagic Diseases and the Physiology of Hemostasis*, Charles C. Thomas, Springfield, Ill., 1942, p. 319, 321.

[5] Quick, A. J., *Am. J. Physiol.*, 1936, **115**, 317.

[6] Quick, A. J., *Lancet*, 1947, **2**, 379.

TABLE III.
Effect of Incubation with Citrated, Oxalated, and Amberlite Plasmas of Man, Dog, and Rabbit on Activity of Full Strength Human Thrombin.
Each figure in the table represents the average clotting time in seconds obtained in several experiments.*

Length of incubation (sec.)		60	120	180	240	300
Human plasma	Amberlite	8	11	13.5	29.5	34
	Citrated	12	14	16	21	29
	Oxalated	12	23	64	450	†
Dog "	Amberlite	8.5	12	18.5	27	52
	Citrated	11	13	17.5	25	34
	Oxalated	13	26.5	49	90	225
Rabbit "	Amberlite	9	11.5	14	22	40
	Citrated	11	12.5	15	20.5	34
	Oxalated	11	35	49	58.5	87

* Equal volumes of full strength human thrombin and of the plasma to be tested were incubated in a water bath at 37°C; the clot was wrapped about a glass rod coated with collodion and removed. After the incubation, 0.1 cc of the mixture were added to 0.2 cc of oxalated homologous plasma as a source of fibrinogen and the clotting time was determined and recorded.
† No clotting in 1 hr.

citrated plasma responded similarly, the behaviour may be linked with the mechanism of decalcification of the 3 agents.

16191 P

Effect of Polyoxyalkylene Sorbitan Monooleate on Blood Cholesterol and Atherosclerosis in Cholesterol-Fed Rabbits.*

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In an attempt to alter the solubility or colloidal stability of cholesterol *in vivo* and thus modify the development of experimental cholesterol atherosclerosis, Hueper[1] administered several commercial detergents to cholesterol-fed rabbits and observed no striking alteration in blood cholesterol levels or degree of atherosclerosis.

This report deals with the effect of oral polyoxyalkylene sorbitan monooleate, Tween 80,‡, a surface-active agent of low toxicity, on the level of blood cholesterol and on the development of atherosclerosis in cholesterol-fed rabbits.

Experimental. Adult male and female rabbits averaging 3.2 kg in weight were fed a stock diet of Rockland rabbit pellets to which were added either:

A. Cholesterol—1 g; peanut oil—3 cc; Tween 80—10 cc.
B. Cholesterol—1 g; peanut oil—3 cc.
C. Tween 80—10 cc.

The ingredients were added separately to the stock diet in individual containers, and thoroughly mixed.

In Experiment 1, 6 rabbits were fed Diet A, and 5 Diet B daily. In Experiment 2, 12

* Aided by a grant from the United States Public Health Service.

† This work was done during the tenure of a Life Insurance Medical Research Fellowship.

1 Hueper, W. C., *Arch. Path.*, 1944, **38**, 381.

‡ Manufactured by the Atlas Powder Co., Wilmington, Del.