amounts of I^{131}. 2. Pituitary tumors result when such radiothyroidectomy is followed by whole-body x-irradiation, or by larger amounts of I^{131}. 3. Similar doses of x-rays alone do not lead to growth of the tumors. It appears that ionizing radiation induces these atypical growths in pituitaries physiologically altered by radiothyroidectomy.

1. Gorbman, A., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 237.

2. Goldberg, R. C., and Chaikoff, I. L., *Endocrinology*, 1951, v48, 1.

3. Furth, J., and Burnett, W. T., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 222.

4. Gorbman, A., *J. Clin. Endocrinol.*, 1950, v10, 1177.

5. Furth, J., Gadsden, E. L., and Burnett, W. T., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 4.

6. Gorbman, A., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 538.

Received August 25, 1952. P.S.E.B.M., 1952, v81.

A Mechanism of Cholesterol Deposition on Arterial Walls. (19874)

JOHAN BJORKSTEN. (Introduced by Edwin L. Gustus.)

From Bjorksten Research Foundation, Madison, Wis.

It is established that cholesterol deposition takes place in the arterial walls of animals (1,2) and man(3,4) and that such deposition is a forerunner of arteriosclerosis and atherosclerosis(5-7). The cause of this cholesterol deposition has not been known(8).

In view of the fact that at least some correlation exists between progressing age and cholesterol deposition in arteries, and in view of the working hypothesis that the fundamental aging reaction is a protein cross-linkage or tanning reaction(9,10) it seemed pertinent to determine whether known cross-linking agents for protein would render arterial wall surfaces selectively adsorptive to cholesterol.

Methods. To this end fresh hog aortas* were slit open. Round test pieces of 20 mm diameter were punched out by means of a cork borer. These test pieces were placed in water solutions of a cross-linking or tanning agent, and control pieces from the same aorta were placed in water. After an interval as shown below, all of these test pieces were immersed in a cholesterol suspension prepared as follows: Fifty g of cholesterol were milled on a rubber mill with submicron particle size acetylene carbon. The object of this was to color the cholesterol particles so that they could be followed visually and photographed. The rubber mill effects a very intimate mixture and complete wetting of the carbon particles by cholesterol. This was verified by microscopic examination. On subsequent dispersion in water, it was found that the carbon particles remain inside the cholesterol particles, and thus are believed not to affect the surface behavior of the cholesterol particles. The cholesterol thus pigmented was added to 300 g of a 4% solution of carboxy methyl cellulose in water, dispersed 10 minutes in a Waring Blendor, and diluted with an additional 200 cc of water. The resultant cholesterol dispersion is quite stable, and can be stored at least for several weeks, though it may be advisable to shake it before use to ensure uniformity.

The aortas were kept immersed in this cholesterol suspension for the periods of time shown below, whereupon they were placed in a 4 inches high by 3⅜ inches wide cylindrical jar containing 100 ml of clean tap water and washed twice by shaking for ½ minute in a shaking machine, ⅛ inch stroke, 450 cycles per minute. The areas which had been in contact with the cross-linking solutions showed heavy cholesterol deposits, while the other areas failed to adsorb cholesterol, and after washing as just stated, were completely free from any traces of this substance. Black

* I am indebted to Oscar Meyer Co. for their cooperation in supplying the aortas fresh from the packing line.

TABLE I. Effect of Certain Cross-Linking Agents on Cholesterol Adsorption by Hog Aorta.

Cross-linking agent	%	Time of immersion in cross-linker	Time of immersion in cholesterol dispersion	Color after wash
Chromic sulfate	10	10 min	10 min	B*
	10	1	10	B
	10	15 sec	10	B in spots only
	1	14 hr	14 hr	B
	.5	14	14	W
Mercuric chloride	10	14	14	W
	1	14	14	W
Lead acetate	3.1	48	10 min	B
	.3	24	10	B
	1	15 min	10	S
	.1	14 hr	10	W
Lead nitrate	.2	14	10	B
	.06	14	10	W
Copper sulfate	5	16	10	B
	1	16	10	S
	.5	16	10	W
Cobalt sulfate	5	16	10	B
	2	16	10	B
	.5	16	10	W
Formaldehyde	37	48	10	W
	3.7	48	10	W
Control, tap water		48	16 hr	W
		24	10 min	W
		12	10	W
		15 min	16 hr	W

* B = black; W = white; S = spotty.

color, when occurring, was due to the carbon inside the suspended cholesterol particles.

Results are summarized in Table I.

It is apparent from this table that *in vitro* the intima of fresh hog aortas may be made to adsorb and retain cholesterol from a carbon-pigmented aqueous cholesterol suspension. This effect is produced by treatment with certain cross-linking agents, of which lead acetate is one of the most efficacious, but copper and chromium salts are also potent. This is not a simple heavy metal effect, for mercuric chloride did not produce this effect even on the drastic test of 48 hours immersion in a saturated solution of mercuric chloride.

Fig. 1 illustrates the sharpness of the adsorption effect obtained with the lead acetate and chromic sulfate, and the total absence of

TABLE II. Reversal of Cholesterol Receptivity of Hog Aorta.

Treatment for attempted reversal of cholesterol receptivity induced by min. effective amount of agents shown in adjacent columns	Exposure time (min)	Chromium sulfate	Cupric sulfate	Cobalt sulfate
Ammonium iodide saturated	10	W†	W	Almost B
Ethylene diamine tetraacetic acid[1]* 10%	10	W	W	B
Choline chloride 2%	10	B	—	—
Disulfhydryl propanol[2] 10%	10	B	B	B
Pepsin .1%[3]	30	Almost W	Almost W	B
Trypsin .1%[4]	30	W	W	B
Ficin .1%[5]	30	W	W	Almost B
Papain .1%[6]	30	Almost W	Almost W	Almost W
Control	0	B	B	B

* [1] Eastman Kodak. [2] BAL in oil, Westcott and Dunning. [3] pH 2, 39°C. [4] pH 8, 39°C. [5] pH 5, 39°C. [6] pH 5, 39°C.

† W = white; B = black.

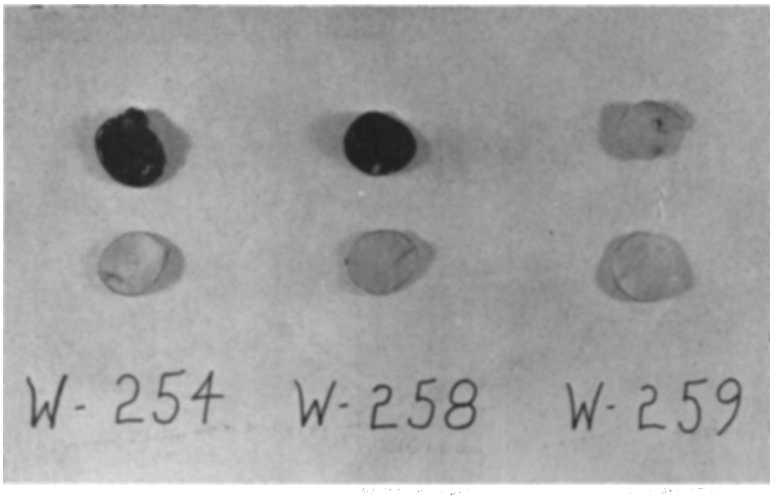

FIG. 1. Representative specimens from Table I. The lower row represents the controls in each case. W254 was treated with chromic sulfate 10% and W258 with lead acetate .3%. W259 was treated with saturated mercuric chloride for a period longer than 10 min and then immersed in the carbon-stained cholesterol suspension and washed.

visible adsorption after treatment with mercuric chloride, or by the untreated controls.

It may be pertinent here to refer to reports that cholesterol adsorption has been induced *in vivo* by uranium salts, which are known as effective protein-tanning agents(11).

It seemed of interest now to determine whether the cholesterol receptivity obtained by the protein cross-linking agents could be reversed. To this end sections of hog aorta were immersed in the cross-linking solutions shown in Table II under conditions which would make them receptive to the cholesterol. The minimum concentration and exposure time to cross-linking agent, shown in Table I, to give cholesterol adsorption, were employed. They were then immersed in treatments which it was felt might reverse the receptive conditions, and then were immersed in the cholesterol test dispersion, washed and observed. Black color of the specimens indicated that the treatment had not reversed the cholesterol receptivity, while white color of the specimens showed that these had been reversed from a cholesterolophilic to a cholesterolophobic condition.

It is seen from this tabulation that the receptive condition brought about with chromium or copper sulfate can be reversed with ethylenediamine tetraacetic acid, ammonium iodide, or proteolytic treatment, but that this is not effective for cobalt sulfate. Disulfhydryl propanol ("British anti-lewisite") is not effective under the conditions tested. Further work is in progress.

These results may justify the working hypothesis that the primary cause of cholesterol deposition and subsequent pathology in arteries may be a chemical process involving the protein material in the lining of the arteries, which changes this from a cholesterolophobic to a cholesterolophilic condition, and that this change may be due to a gradual cumulative action of certain protein cross-linking agents. It is certainly extremely suggestive that the loss of elasticity of the arterial wall, and its ability to adsorb cholesterol, may be related by so simple an hypothesis as the chemical change in proteins induced by cross-linking or tanning agents.

Summary. Certain substances reactive with proteins have been found *in vitro* to cause selective adsorption of suspended cholesterol to the inner lining of hog aorta.

1. Wacker, L., and Heck, W., *München Med. Wochensch.*, 1913, v60, 2097.

2. ———, *Arch. exp. Path. u. Pharmakol.*, 1913, v74, 416.

3. Meeker and Jobling, *Arch. Path.*, 1934, v18, 252.

4. Learn, T., *Arch. Path.*, 1944, v37, 16.

5. Duff, G. L., *Arch. Path.*, 1935, v20, 81, 259.

6. Katz, L. N., and Dauber, D. V., *J. Mt. Sinai*

Hosp., 1945, v12, 382.

7. Lansing, A. I., Alex, M., and Rosenthal, T. B., *J. Gerontology*, 1950, v5, 112.

8. Kendall, F. E., *Chem. and Eng. News*, 1952, v30, 2598.

9. Bjorksten, J., and Champion, W. J., *J. Am. Chem. Soc.*, 1942, v64, 868.

10. Bjorksten, J., *Advances in Protein Chem.*, 1951, v6, 358.

11. Campbell, M., and Longebaugh, G. M., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 92.

Received August 25, 1952. P.S.E.B.M., 1952, v81.

Microdetermination of Calcium in Blood Serum by Direct Titration. (19875)

ANDRE C. KIBRICK,* M. ROSS, AND HANNAH E. ROGERS.†

From the Department of Chemistry, Bronx Hospital, New York.

The microdetermination of calcium in serum by rapid titration with a chelating reagent, ethylene diamine tetracetate(1), has obvious advantages. In the method of Sobel and Hanok(2), in which the dye, eriochrome black T, is used as indicator, magnesium is also titrated, and the end-point is obscured in icteric and hemolyzed serum. Greenblatt and Hartman(3) propose a method with murexide (ammonium purpurate) as indicator, but do not mention interference by magnesium. Although they present data to show that the results obtained in serum, urine and spinal fluid agree with those obtained by a standard method, the end-point has been found so poor in this laboratory that we have not been able to titrate serum with the naked eye. We also have evidence(4) that it is not possible to titrate calcium in urine in this way.

In this paper we describe a method for the microtitration of calcium in serum in a photoelectric colorimeter with ethylene diamine tetracetate and murexide as indicator. Magnesium is not titrated in this method, and neither hemolyzed nor icteric blood interferes in the titration. In a later paper we will describe a macromethod for the titration of calcium in urine.

Experimental. We have used a Lumitron photoelectric colorimeter with an adaptor to hold tubes of 15 mm diameter. The solutions were titrated in Klett-Summerson microcolorimeter tubes while the tubes were in the colorimeter, and the colorimeter was read continuously during titration. Although this arrangement has been found most convenient, any colorimeter with an adaptor to hold tubes of this size, and any short tube of about 15 mm diameter should be suitable. A convenient burette was made by sealing with De Khotinsky cement a 4 inch hypodermic needle of about 18 gauge bore to the tip of a 0.2 ml Kahn pipette graduated in thousandths. The needle was bent at a right angle so that at least 3 inches of needle was perpendicular to the pipette, and the point of the needle was filed off straight. A piece of rubber tubing to serve as a reservoir for mercury was attached to the other end of the pipette, and the open end of the tubing was plugged with a glass rod. This burette was mounted on a horizontal wooden stand which also carried a screw clamp to propel the mercury and titrating fluid, and another bent needle which delivered nitrogen gas from a tank to mix the solution in the tube during titration. Our stand was so constructed that the entire burette assembly could be raised and swivelled at the start and at the end of each titration at the colorimeter.

Reagents. 1) Concentrated stock calcium standard solution. Dissolve 2.497 g of dry calcium carbonate with a minimal amount of hydrochloric acid. Neutralize with sodium hydroxide, and dilute to 500 ml. 2) Working standard calcium solution. Dilute 5 ml of the concentrated stock solution to 100 ml. 100 ml = 10 mg Ca. 3) Saturated picric acid

* Present address: Department of Chemistry, New York University College of Medicine, New York.

† Present address: Department of Pathology, Cornell University Medical School, New York.