dence has been presented to the contrary(7). Immune hemolysis and tissue immune reactions, however, differ in several respects(2). Indeed, Keller(14) has reported formation of lysolecithin in rat mast cells during antigen-antibody reaction *in vitro*. If lysolecithin were formed during tissue immune reactions, the evidence presented here indicates that it could contribute significantly to tissue damage. It could also account for edema production, as seen with urticaria and angioneurotic edema. The potent cytotoxic effect of lysolecithin, in addition, could account in large part for the deep necrosis often seen at the site of a snakebite.

Summary. A series of mice was injected subcutaneously with lysolecithin and the tissues examined at measured intervals. Within 10 minutes after injection, striking edema with necrosis of fat tissue was evident at the injection site. Subsequent changes included infiltration with neutrophil leukocytes and histiocytes and hyalinization and necrosis of muscle cells. Despite the marked edema and contrary to previous reports, vascular damage was not demonstrated by light microscopy. The production of lysolecithin could account for much of the local necrosis resulting from snakebite.

1. Mongar, J. L., Schild, H. O., Physiol. Rev., 1962, v42, 226.
2. Austin, K. F., Humphrey, J. H., Adv. Immunol., 1963, v3, 1.
3. Uvnäs, B., Ann. N. Y. Acad. Sci., 1963, v103, 278.
4. Phillips, G. B., Middleton, E., Jr., Proc. Soc. Exp. Biol. and Med., 1965, v119, 465.
5. Zeller, E. A., in Sumner, J. B., Myrbäck, K., The Enzymes, Academic Press, Inc., N. Y., 1951, 986.
6. Phillips, G. B., Biochem. Biophys. Acta, 1958, v29, 594.
7. Phillips, G. B., Middleton, E., Jr., J. Immunol., 1965, v94, 40.
8. Middleton, E., Jr., Phillips, G. B., J. Lab. & Clin. Med., 1964, v64, 889.
9. Högberg, B., Uvnäs, B., Acta Physiol. Scand., 1957, v41, 345.
10. Feldberg, W., Holden, H. F., Kellaway, C. H., J. Physiol. (Lond.), 1938, v94, 232.
11. Belfanti, S., Z. für Immunitatsf., 1925, v44, 347.
12. Guerrini, G., ibid., 1925, v45, 249.
13. Fisher, V. H., Haupt, I., Z. Naturforsch., 1961, v16B, 321.
14. Keller, R., Helv. Physiol. et Pharm. Acta, 1962, v20, c66.

Received April 19, 1965. P.S.E.B.M., 1965, v119.

Effect of Defatted Brain Extract and Soy Sterols on Plasma Cholesterol Levels and Atherogenesis in Cholesterol-Oil Fed Cockerels.* (30318)

R. PICK,† S. JAIN, C. KAKITA AND P. JOHNSON
(Introduced by L. N. Katz)
Cardiovascular Institute, and Department of Cardiovascular Disease, Division of Medicine, Michael Reese Hospital and Medical Center, Chicago, Ill.

It has long been inferred that elevated serum cholesterol levels are positively correlated with degree of atherosclerosis, both in animals and man. Consequently, intensive efforts have been made to devise means to reduce serum cholesterol levels in the hope of preventing atherosclerosis. The search goes on for an ideal hypocholesterolemic agent which would be practical to administer and harmless even on extended use.

Brain extract (cerebroside), and sitosterols from plants have been reported by various workers to have a favorable hypocholesterolemic effect both in clinical trials and in animal studies(1-6). The present study further evaluates the status of these products in re-

* This work was supported in part by grant HE-06375 from Nat: Heart Inst.

† Studies were carried out, in part, during Dr. Pick's tenure as an Established Investigator, American Heart Assn., supported by the Chicago and Illinois Heart Assn.

ducing serum cholesterol levels and atherosclerosis in cholesterol-oil fed cockerels.

Material and methods. The established technics of this Department were followed throughout. Young growing cockerels were used. Four experiments were carried out. In Exp. 1, 2 and 3 the atherogenic ration, used for all birds, consisted of chick starter mash supplemented with 1% cholesterol and 5% cottonseed oil. In Exp. 4 chick starter mash alone without supplement was used.

Plasma cholesterol analyses(8) were carried out in all the birds at the end of 2 and 5 weeks of the experimental period, and plasma phospholipids(9) at the end of 5 weeks. All the cockerels were killed and autopsied after 5 weeks on the experimental diet. The aortas were removed and cut open longitudinally. The degree of aortic atherosclerosis was graded into 5 groups (0 to 4) according to the degree of elevation of the lesions and the area of the intima involved by atheroma. For this evaluation only thoracic aorta atherosclerosis was evaluated (including the brachiocephalic arteries). The abdominal aorta was not included because it is markedly susceptible to spontaneous atherosclerosis in the cockerel.

The hearts were removed and fixed in formalin. Two blocks were taken from each heart and frozen sections were cut from each of the blocks and stained with Sudan IV and hematoxylin. A count was made of the total number of arteries and of those that showed sudanophilic intimal thickening or plaques. Not infrequently the intima or media of a vessel retained some Sudan IV but showed no structural abnormality. This was regarded as lipid infiltration only and not considered as evidence of atherosclerosis. The percentage of arteries with lesions, as defined, was used as the index of coronary atherosclerosis.

The experimental design was as follows:

Experiment 1 (Series 26, 28 and 37).

Group A—Atherogenic diet.

Group B—Atherogenic diet + 5% cerebroside.‡

Experiment 2 (Series 28), essentially a probe to determine if results in experiment 1 were dose dependent.

Group A—Atherogenic diet.

Group B—Atherogenic diet + 3% cerebroside.

Group C—Atherogenic diet + 5% cerebroside.

Group D—Atherogenic diet + 10% cerebroside.

Experiment 3 (Series 19, 23, 28 and 31).

Group A—Atherogenic diet.

Group B—Atherogenic diet + 5% soy sterol.§

Experiment 4 (Series 42 and 67), done to determine if soy sterols have an effect on endogenous hypercholesterolemia induced by estrogens. Estrogens were given as mixed conjugated equine estrogens (Premarin) in the drinking water, 25 mg/chick/day.§

Group A—Chick starter mash + estrogens.

Group B—Chick starter mash + estrogens + 5% soy sterol.

Results. Cerebrosides—(Exp. 1 and 2). As shown in Table I, the cockerels given 5% cerebrosides in their atherogenic diet had significantly lower serum cholesterol levels, serum phospholipids and C/P ratios compared with the cockerels given only the atherogenic diet. They also showed a significantly lower degree of atherosclerosis both in the thoracic aorta and the coronary arteries.

As can be seen from Fig. 1, the hypocholesterolemic and anti-atherogenic effects of cerebrosides were directly proportional to the dose exhibited. However, the chicks given the highest dose of cerebrosides (10%) did not thrive as well; their body weights were lower than in the other groups.

These results are in accord with those of Gordon *et al* in chicks(1), Rosenheim and Webster in rats and dogs(2), and Jones *et al* in chicks(3) and in man(10). However, none of the preceding studies dealt with the effect of cerebrosides on coronary atherosclerosis.

There is evidence to suggest that the cerebrosides in some way bind the intraintestinal

‡ We are grateful to Dr. E. Levin of the Viobin Corp. for the supply of cerebrosides.

§ We are grateful to Dr. P. L. Julian of the Glidden Co. for the supply of soy sterols, and to Dr. J. B. Jewell of Ayerst Laboratories for the supply of Premarin.

cholesterol, or otherwise inhibit reabsorption into the portal system. This leads to an increase in the turnover rate of cholesterol, and if this is elevated, a reduction in its level(11).

Soy sterols—(Exp. 3 and 4). As shown in Table II, the group of chickens given 5% soy sterols showed serum cholesterol levels (171 mg %) within the normal range in spite of the atherogenic diet. This was in sharp contrast to the marked hypercholesterolemia (1302 mg %) in the group given the same diet without soy sterol ($p < .001$). The difference in the levels of serum phospholipids and C/P ratios between the two groups was not significant. Soy sterols were also able almost completely to inhibit atherogenesis in

TABLE I. Effect of Feeding Cerebrosides on Serum Cholesterol Levels and Atherosclerosis in Chicks on Cholesterol-Oil Supplemented Mash* (S26, 28. 37).

Group	Total No. of birds	Serum cholesterol (C), mg %	C/P† ratio	Thoracic aorta atherosclerosis			Coronary atherosclerosis		
				No. of birds with lesions		Avg grade of lesions	No. of birds with lesions		% Arteries with lesions
				Incidence	%		Incidence	%	
A—atherogenic diet	29	507 ± 50‡	1.48 ± .13	17	59	.72 ± .14	23	82	11.6 ± 1.8
B—atherogenic diet + 5% cerebroside	29	309 ± 24	1.04 ± .08	10	34	.25 ± .08	19	66	5.8 ± 1.0
			p values						
Group A *vs* Group B	—	<.001	<.01	—	—	<.01	—	—	<.01

* This consisted of chick starter mash supplemented with 1% cholesterol and 5% cottonseed oil.
† P is serum phospholipid in mg %.
‡ ± is standard error of mean.

TABLE II. Effect of Feeding Soy Sterols on Serum Cholesterol Levels and Atherosclerosis in Chicks on Cholesterol-Oil Supplemented Mash* (S19, 23, 28, 31).

Group	Total No. of birds	Serum cholesterol (C), mg %	C/P† ratio	Thoracic aorta atherosclerosis			Coronary atherosclerosis		
				No. of birds with lesions		Avg grade of lesions	No. of birds with lesions		% Arteries with lesions
				Incidence	%		Incidence	%	
A—atherogenic diet	62	1302 ± 17‡	1.94 ± .06	62	100	1.52 ± .08	57	92	22.1 ± 1.5
B—atherogenic diet + 5% soy sterols	61	171 ± 8	1.20 ± .06	15	24	.91 ± .05	11	18	2.4 ± .7
			p values						
Group A *vs* Group B	—	<.001	—	—	—	<.001	—	—	<.001

* This consisted of chick starter mash supplemented with 1% cholesterol and 5% cottonseed oil.
† P is serum phospholipid in mg %.
‡ ± is standard error of mean.

TABLE III. Effect of Feeding Soy Sterols on Serum Cholesterol Levels and Atherosclerosis in Chicks Given Estrogens* (S42, 67).

Group	Total No. of birds	Serum cholesterol (C), mg %	C/P† ratio	Thoracic aorta atherosclerosis: No. of birds with lesions, Incidence	Thoracic aorta atherosclerosis: No. of birds with lesions, %	Thoracic aorta atherosclerosis: Avg grade of lesions
A—Basal diet‡ + estrogens	19	285 ± 34§	.28 ± .03	9	47	.32 ± .10
B—Basal diet + estrogens + 5% soy sterols	16	324 ± 50	.28 ± .02	9	56	.33 ± .09
		p values				
Group A *vs* Group B	—	N.S.‖	N.S.	—	—	N.S.

* Estrogens were given as Premarin® in drinking water 25 mg/chick/day.
† P is serum phospholipid in mg %.
‡ Basal diet consisted of chick starter mash without any supplement.
§ ± is standard error of mean.
‖ N.S. = statistically not significant.

the thoracic aorta as well as in the coronary arteries.

A marked reduction in serum and liver cholesterol levels and thoracic aorta atherosclerosis attributed to corn sterols, B sitosterols or soy sterols when fed with an atherogenic diet was also reported in chicks by Peterson(4) and by Fisher *et al*(12), in rabbits by Pollak(13), and in man by Pollak(5) and by Best *et al*(6).

The effect of soy sterols was different when the hypercholesterolemia was of endogenous origin, induced by estrogens (Table III). Here soy sterols had practically no effect either on the levels of serum cholesterol, phospholipids and C/P ratio, or on the degree of aortic atherosclerosis. The coronary arteries, as expected, were free of atherosclerosis, whether or not soy sterols were given, because of the exhibition of the estrogens(14).

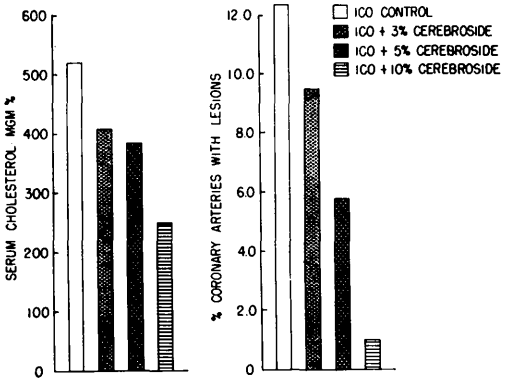

FIG. 1. Relationship between dose of cerebroside in the diet and serum cholesterol levels and coronary atherogenesis in cholesterol-oil fed cockerels (S28).

As regards the mode of action of the sitosterols, Hernandez, *et al*(15) reported that when soy sterols and B sitosterols were fed simultaneously with cholesterol-14-C^{14} *via* stomach tube to rats, there was a marked drop in the C^{14} recoveries from the thoracic duct. Davis(16) has suggested that cholesterol and sitosterol form a mixed crystal in the gastrointestinal tract which is much less soluble than either alone thus preventing its absorption. This observation would also explain the absence of any significant hypocholesterolemic effect in our chicks when the hypercholesterolemia was endogenous in origin.

The results of this study clearly demonstrate that agents which can effectively suppress the serum cholesterol levels also effectively inhibit the development of coronary atherosclerosis in chicks. This conclusion does not preclude other modes of action on atherosclerosis independent of serum cholesterol level. Hypocholesterolemic agents, however, may be presumed to be anti-atherogenic unless evidence develops to the contrary in a particular instance.

Conclusion. Both cerebrosides and soy sterols were very effective in reducing serum cholesterol levels, and thoracic aorta and coronary atherosclerosis in chicks fed an atherogenic diet. This effect of cerebrosides was directly proportional to the dose administered. Soy sterols were without effect on endogenously induced hypercholesterolemia produced by estrogens.

We are indebted to Dr. J. Stamler for his cooperation in some of the early studies.

1. Gordon, R. B., Kraft, S. C., Jones, R. J., J. Lab. and Clin. Med., 1953, v41, 583.
2. Rosenheim, O., Webster, R. A., J. Biol. Chem., 1941, v35, 920.
3. Jones, R. J., Kraft, S. C., Huffman, B. A., Balter, E. L., Gordon, R. B., Circ. Res., 1953, v1, 530.
4. Peterson, D. W., Proc. Soc. Exp. Biol. and Med., 1951, v78, 143.
5. Pollak, O. J., Circulation, 1953, v7, 702.
6. Best, M. M., Duncan, C. H., Van Loon, E. J., Wathen, J. D., ibid., 1954, v10, 201.
7. Katz, L. N., Stamler, J., Experimental Atherosclerosis, Charles C Thomas, Springfield, Ill., 1953.
8. Schoenheimer, R., Sperry, W. M., J. Biol. Chem., 1934, v106, 754.
9. Zilversmit, D. B., Davis, A. K., J. Lab. & Clin. Med., 1950, v35, 155.
10. Jones, R. J., ibid., 1956, v47, 261.
11. Jones, R. J., Reiss, O. K., Balter, E. L., Cohen, L., Proc. Soc. Exp. Biol. and Med., 1957, v96, 442.
12. Fisher, H., Weiss, H. S., Griminger, P., ibid., 1963, v113, 415.
13. Pollak, O. J., Circulation, 1953, v7, 696.
14. Pick, R., Stamler, J., Rodbard, S., Katz, L. N., ibid., 1952, v6, 276.
15. Hernandez, H. H., Peterson, D. W., Chaikoff, I. L., Dauben, W. G., Proc. Soc. Exp. Biol. and Med., 1957, v96, 498.
16. Davis, W. W., Trans. N. Y. Acad. Sci., 1955, v18, 123.

Received April 19, 1965. P.S.E.B.M., 1965, v119.

A New Method for Detection of "Immune" Type of Anti-B Antibody.* (30319)

MITSUO YOKOYAMA AND ANN STEGMAIER (Introduced by Albert B. Benedict)
Department of Genetics, University of Hawaii, Honolulu

Winstanley *et al*(1) and Konugres *et al*(2) established a method for detection of the "immune" anti-A antibody using certain pig red blood cells (A^p), and demonstrated the antibody by agglutination and hemolysis of the A^p red cells which possess human A-like antigen. Tovey *et al*(3) employed A^p red cells for prenatal diagnosis of the hemolytic disease of the newborn due to ABO incompatibility. These investigators used the method of antibody-absorption (A-A test); that is, the absorption of the immune anti-A antibody, but not the naturally occurring antibody with A^p red cells. Subsequently Yokoyama *et al*(4) and Polley *et al*(5) showed the usefulness of the A^p system for detection of immune anti-A antibody. Recently the A^p antigen was classified into 3 classes and the A^{p1} red cells were found to detect "immune" anti-A activity with a more definite reaction by agglutination and hemolysis than the A^{p2} and A^{p3} red cells(6). Yokoyama and Fudenberg(7) also reported that the anti-A^p activity occurred in the γ_2 globulin (IgG), γ_1A (IgA) and β_2M (IgM) fractions.

A similar method for detecting the immune anti-B antibody, which is different from naturally occurring anti-B antibody, has long been sought, with the intention of utilizing the method in pre- and post-natal diagnosis of hemolytic disease of the newborn due to an incompatible pregnancy involving the B antigen. The present paper describes a new method for detecting the immune anti-B antibody.

Materials and methods. Blood samples from 30 New Zealand white rabbits, between the ages of 2 and 7 months, were mixed with an equal volume of Alsever's solution. Prior to use the red cells were washed 4 times with physiological saline. The red cells from each rabbit were tested against blood grouping sera developed against rabbit rbc isoantigens.† A 2% red cell suspension in saline was mixed with an equal volume of antiserum and incu-

* Genetics Paper No. Pacific Biomedical Research Center Contribution No.

† Anti-A, anti-D and anti-F obtained through the courtesy of Dr. Carl Cohen, Dept. of Biology, Western Reserve Univ., Cleveland, Ohio.