jection into mice. The nontype-specific carbohydrate, after decomposition by either strain, also failed to induce purpura.

7163 P

Production of Lens Sensitivity in Rabbits by the Action of Staphylococcus Toxin.

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Extracts of lens[1] derived from any of the vertebrates, with the exception of rabbit lens, produce, when injected into rabbits, precipitins for all lens extracts Although a high precipitin titer can be developed neither an Arthus reaction nor an anaphylactic state can be produced in the rabbit. In a previous report[2] it was shown that Staphylococcus toxin, injected intracutaneously into rabbits, produces not only an antitoxin but also a hypersensitive state to the beef bouillon in which the toxin is produced and to beef serum. This result suggested that if such a poor antigen as beef broth could be made antigenically active, a similar antigenic effect could be developed for lens substance if this material were added to the broth. Accordingly the following experiments were undertaken.

Beef eyes, trimmed of all extraocular tissue, were dropped into boiling water for 30 seconds. With a sterile scissors a transverse corneal incision was made and the lens expressed into a tube containing 20 cc. of hormone bouillon. After a preliminary incubation period of 7 days to eliminate the contaminated tubes, the sterile tubes were inoculated with toxin-forming *Staph. aureus,* strain Ha. After 10 days' incubation at 37°C. the contents of the tubes were pooled, ½% trikresol added and filtered through a Berkefeld V filter. Injected intravenously, 0.3 cc. killed a 3000 gm. rabbit within 24 hours. This lens broth toxin precipitated with lens immune serum in dilutions as high as 1-50,000.

Rabbits were injected intracutaneously with 0.1 cc. amounts of lens broth toxin and lens extract, each in a different skin area at intervals of one week. Two rabbits were injected intravenously with

[1] Woods, A. C., Allergy and Immunity in Ophthalmology, Johns Hopkins Press, Baltimore, 1933. Review of the literature on immunology of the lens.

[2] Burky, E. L., *J. Immunol.,* 1933, **25**, 419.

5 cc. amounts of lens extracts once a week. Other details of technique have been outlined elsewhere.[1, 2]

Beef, swine and rabbit lens extracts produced areas of swelling, erythema and occasional necrosis greater than 2x2 cm. in 8 of 9 rabbits after the 3rd or 4th injection of lens broth toxin. The ninth rabbit did not become sensitive to lens until after the 6th injection. Preceding this experiment 4 rabbits were repeatedly injected intracutaneously with mixtures of beef serum and lens extract without developing sensitivity or precipitins for lens extract although a marked Arthus reaction to beef serum developed. The intravenously injected rabbits were not skin reactive after the 5th intravenous injection of lens extract.

TABLE I.

No. Rabbits	No. Injections	Material injected	Skin Reactions to Lens Extract	Precipitin titers Dilution of lens	Intraocular Reactions
8	3	Lens toxin, .1 cc. Lens extract, 1 cc. intracutaneous	Positive after 3rd or 4th injection	1-50,000 or more	Positive See text
1	6	Lens toxin, .1 cc. Lens extract, .1 cc. intracutaneous	Negative after 4th injection	1-5000	Negative
2	5	Lens extract, 5 cc. intravenous	Negative	1-5000	"
4		Normal controls	Not done	Negative	"
4	8	Beef serum + lens extract, 0.4 cc. intracutaneous	Negative	"	"

The 9 rabbits in the intracutaneous group and the 2 rabbits in the intravenous group developed precipitins for lens extract. The highest titers were found in the sensitive animals (1-50,000 or more). The intravenously injected animals after 5 injections had titers not higher than 1-5,000. Lens precipitins were not present in these serums before immunization was begun.

To determine whether these skin reactions had any real significance the 11 rabbits and 4 normal controls were treated in the following manner after the 4th intracutaneous injection. Under cocaine anesthesia, a 25 G. needle attached to a syringe was inserted into the anterior chamber and about 0.2 cc. of aqueous humor was drawn into the syringe and then injected into the lens.

The sensitive animals developed marked intraocular inflammation

cornea with vascularization, positive aqueous ray and plastic iritis. The ocular inflammation continued for at least 7 days in the eyes not removed for histological study before this time. The immune but insensitive animals reacted like the normal controls with a purely traumatic reaction that subsided in less than 7 days.

Summary. Rabbits cannot be sensitized to lens protein by ordinary methods. By the intermediary action of Staphylococcus toxin a skin and ocular sensitivity to lens substance has been developed. Precipitin titers, higher than those developed by ordinary methods, are also produced. This method, to our knowledge, has not been previously reported and may be of wider application in the experimental study of hypersensitive states.

A more complete report will be published in an ophthalmological journal.

7164 C

Phosphatase Activity of the Bones and Kidneys in Thyrotoxicosis.*

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Considerable experimental work has been done within the past few years on the phosphatase activity of various bodily tissues. Robison[1] showed that soluble salts of hexosemonophosphoric esters were hydrolyzed by an enzyme present in ossifying cartilage, kidney, and the shafts of long bones. Kay,[2] Robison,[1] and others have shown that the intestinal mucosa, kidney, and whole bone contain the greatest concentration of this enzyme. Furthermore, the plasma and tissue phosphatase activity has been shown to vary from the normal in certain pathological conditions. Page[3] has shown that bone phosphatase is diminished if an excess of vitamin D is fed to an animal, along with the coincident decalcification of bone and metastatic calcification of other tissues. Parathormone has also been shown[4] to

* This is reprint No. 324 of the Cancer Commission of Harvard University.

[1] Robison, R., *Biochem. J.*, 1923, **17**, 286.

[2] Kay, H. D., *Biochem. J.*, 1928, **22**, 855.

[3] Baumgartner, L., King, E. J., and Page, I. H., *Biochem. Z.*, 1929, **213**, 170.

[4] Page, I. H., and Reside, M., *Biochem. Z.*, 1930, **223**, 171; 1930, **226**, 273. Page, I. H., *Biochem. Z.*, 1930, **223**, 222.